CERTIFICATE OF EXPRESS MAIL (37 CFR 1.10) Label No. EH386303945US

I hereby certify that the attached papers and/or fee are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service on the date shown below in an envelope addressed to Box PCT, Assistant Commissioner for Patents, Washington D.C. 20231.

Feb. 15, 2001 (Date) | Mary Rutkowski (Printed Name) | Mary Rutkowski (Signature)

FORM PTO-1390 (REV 12/29/99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: PPD 50355/UST

U. S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/763019

INTERNATIONAL APPLICATION NO.:	INTERNATIONAL FILING DATE:	PRIORITY DATE CLAIMED:
PCT/GB99/02720	August 17, 1999	August 18, 1998

TITLE OF INVENTION: **Polynucleotide Sequences**

EXPRESS MAIL NO.: **EH386303945US**

APPLICANT(S) FOR DO/EO/US: **Ian EVANS and John RAY**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND or SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1)).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.

    ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Information Disclosure Statement, Form PTO-1449, Copies of 4 refs. cited on PTO-1449, Paper Copy of Sequence Listing, Computer Disk Containing Sequence Listing, Statement to Support Filing and Submission under 37 CFR 1.821-1.825

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/763019		PPD 50355/UST

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1. 492 (a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . . . $ 1000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . . .$ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. . .$ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) . . . . . .$ 690.00

☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). . . . . .$ 100.00

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$ 860.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	76-20 =	56	x $18.00	**$1,008.00**	
Independent claims	8 - 3 =	5	x $80.00	**$ 400.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	**$ 270.00**	
			TOTAL OF ABOVE CALCULATIONS =	**$2,538.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				**$**	
			SUBTOTAL =	**$2,538.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +					
			TOTAL NATIONAL FEE =	**$2,538.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +					
			TOTAL FEES ENCLOSED =	**$2,538.00**	
				Amount to be refunded:	$
				Charged	**$**

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **09-0013** in the amount of **$ 2,538.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **09-0013**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Syngenta Crop Protection, Inc.
Intellectual Property Section
2 Righter Parkway
P.O. Box 15458
Wilmington, DE 19850-5458

23698

PATENT TRADEMARK OFFICE

Liza D. Hohenschutz Feb. 15, 2001
SIGNATURE DATE

Liza D. Hohenschutz
NAME

33,712
REGISTRATION NUMBER

(302)476-2088
TELEPHONE NUMBER

CERTIFICATE OF EXPRESS MAIL (37 CFR 1.10) Label No. EH386303945US

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Feb. 15, 2001 (Date) | Mary Rutkowski (Printed Name) | Mary Rutkowski (Signature)

Docket No. PPD 50355/UST

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF.: Ian EVANS et al.

SERIAL NUMBER: Not Assigned

GROUP ART UNIT: Not Assigned

FILED:

EXAMINER: Not Assigned

FOR: Polynucleotide Sequences

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 CFR §§1.821 THROUGH 1.825

☒ I hereby state, in accordance with the requirements of **37 C.F.R. §1.821(f)**, that the information recorded in computer readable form is identical to the written Sequence Listing.

☒ I hereby state that the submission filed in accordance with **37 C.F.R. 1.821(g)** does not include new matter.

☐ I hereby state that the submission filed in accordance with **37 C.F.R. §1.821(h)** does not include new matter or go beyond the disclosure in the international application as filed.

☒ I hereby state that the amendments, made in accordance with **37 C.F.R. §1.825(a)**, included in the substitute sheet(s) of the Sequence Listing are supported in the application, as filed, at pages 1-23 of the Sequence Listing. I hereby state that the substitute sheet(s) of the Sequence Listing does (do) not include new matter.

☒ I hereby state that the substitute copy of the computer readable form, submitted in accordance with **37 C.F.R. §1.825(b)**, is the same as the amended Sequence Listing.

☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with **37 C.F.R. §1.85(d)**, contains identical data to that originally filed.

Respectfully submitted,
Syngenta Crop Protection, Inc.

Liza B. Hohenschutz
Attorney for Applicant(s)
Reg. No. 33,712
Telephone: 302/476-2088

Dated: February 15, 2001

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Feb. 15, 2001 (Date) | Mary Rutkowski (Printed Name) | Mary Rutkowski (Signature)

PATENT APPLICATION
PPD 50355/UST

IN THE UNITED STATES ELECTED OFFICE

INTERNATIONAL APPLICATION NO.: PCT/GB99/02720

INTERNATIONAL FILING DATE: August 17, 1999

PRIORITY DATE CLAIMED: August 18, 1998

APPLICANTS: Ian EVANS *et al.*

TITLE: POLYNUCLEOTIDE SEQUENCES

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Prior to assigning a serial number and calculating the filing fee, please amend the above-identified application as follows:

IN THE CLAIMS

Claim 8, line 1, delete "preceding claim" and substitute therefor --one of claims 1 to 6--.

Claim 9, line 1, delete "preceding claim" and substitute therefor --one of claims 1 to 6--.

Claim 10, line 2, delete "of the preceding claims" and substitute therefor --any one of claims 1 to 6--.

Claim 11, lines 1 and 2, delete "claims 1 to 9 or the vector of claim 10" and substitute therefor --claims 1 to 6--.

Claim 12, lines 1 and 2, delete "the preceding claim" and substitute therefor --claim 11--.

13. (amended) The progeny of the plants of claim 12 [the preceding claim, which progeny comprises the polynucleotide of any one of claims 1 to 9 stably incorporated into its genome and heritable in a mendelian manner], the seeds of such plants and such progeny.

Claim 14, line 4, delete "9 or the vector of claim 10" and substitute therefore --6--.

Claim 15, line 1, delete "9, or the vector of claim 10" and substitute therefor --6--.

Claim 18, line 2, delete "either of claims 12 or 13" and substitute therefor --claim 12.

Claim 19, line 1, delete "9, or the vector of claim 10" and substitute therefore --6--.

REMARKS

The claims have been amended to remove improper multiple dependencies and place them in a better form for examination.

An early and favorable Office Action is requested.

Respectfully submitted,
Syngenta Crop Protection, Inc.

Liza D. Hohenschutz

Liza D. Hohenschutz
Reg. No. 33,712
Attorney for Applicants

Dated: February 15, 2001

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Feb. 15, 2001 (Date) | Mary Rutkowski (Printed Name) | Mary Rutkowski (Signature)

PATENT APPLICATION
PPD 50355/UST

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF.: Ian Evans et al.

SERIAL NUMBER: Not Assigned

GROUP ART UNIT: Not Assigned

FILED:

EXAMINER: Not Assigned

FOR: Polynucleotide Sequences

SECOND PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Prior to a first Official Action, please amend the above-identified patent application as follows:

IN THE SEQUENCE LISTING:

Delete the Sequence Listing (pages 1-23) and insert the Sequence Listing submitted herewith.

REMARKS

Applicants submit a paper copy of the Sequence Listing in compliance with 37 CFR 1.821-1.825, a computer disk containing the Sequence Listing in computer readable form and a Statement to Support Filing and Submission in Accordance with 37 CFR 1.821-1.825.

An early and favorable Office Action is requested.

Respectfully submitted,
Syngenta Crop Protection, Inc.

Liza D. Hohenschutz
Reg. No. 33,712
Attorney for Applicants

Dated: February 15, 2001

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POLYNUCLEOTIDE SEQUENCES

The present invention relates to recombinant DNA technology, and in particular to nucleotide sequences (and expression products thereof) which are used in the production of transgenic plants.

The present invention provides, inter alia, nucleotide sequences useful in the production of plants which show improved resistance to infection by microorganisms such as bacteria and fungi.

According to the present invention there is provided a polynucleotide comprising a sequence selected from those depicted in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4 and SEQ ID No. 5.

Also included within the invention is the translation product of the said polynucleotide sequences depicted in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4 and SEQ ID No. 5.

The invention further provides a polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 53 to 385 in SEQ ID No. 1, nucleotides 11 to 334 in SEQ ID No. 2, nucleotides 24 to 317 in SEQ ID No. 3, nucleotides 20 to 343 in SEQ ID No. 4 or nucleotides 1 to 446 in SEQ ID No. 5. Also included within the invention is the translation product of the region comprised by nucleotides 53 to 385 in SEQ ID No. 1, by nucleotides 11 to 334 in SEQ ID No. 2, by nucleotides 24 to 317 in SEQ ID No. 3 or by nucleotides 20 to 343 in SEQ ID No. 4 or nucleotides 1 to 446 in SEQ ID No. 5 and protein having an amino acid sequence which is at least 85% similar to said product. The said translation product is a preproprotein comprising a signal sequence, protein encoding sequence and C-terminal propeptide which is naturally processed to yield mature biologically active protein.

The invention further provides a polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 137 to 286 in SEQ ID No. 1, nucleotides 95 to 244 in SEQ ID No. 2, nucleotides 108 to 257 in SEQ ID No. 3, nucleotides 104 to 253 in SEQ ID No. 4 or nucleotides 177 to 326 in SEQ ID No. 5. These polynucleotide sequences are especially preferred. Also included within the invention and especially preferred is the translation product of the region comprised by nucleotides 137 to 286 in SEQ ID No. 1, by nucleotides 95 to 244 in SEQ ID No. 2, by nucleotides 108 to 257 in SEQ ID

No. 3 or by nucleotides 104 to 253 in SEQ ID No. 4 and protein having an amino acid sequence which is at least 95% similar to said product. The translation product is an antimicrobial protein. The related antimicrobial proteins DmAMP1 and Dm-AMP2 have been described in Published International Patent Application No. WO 93/05153 and in Osborn et al (1995) FEBS Lett. 368 257-262.

The invention further provides a polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 287 to 385 in SEQ ID NO. 1, nucleotides 245 to 334 in SEQ ID No. 2, nucleotides 258 to 317 in SEQ ID No. 3, nucleotides 254 to 343 in SEQ ID No. 4 or nucleotides 327 to 446 in SEQ ID No.5. These nucleotides are particularly preferred according to the invention and encode protein sequences which may be used as cleavable linkers in the co-expression of multiple proteins as is described further herein. The invention further extends to the translation product of nucleotides 287 to 385 in SEQ ID NO. 1, nucleotides 245 to 334 in SEQ ID No. 2, nucleotides 258 to 317 in SEQ ID No. 3, nucleotides 254 to 343 in SEQ ID No. 4 or nucleotides 327 to 446 in SEQ ID No.5 and protein having an amino acid sequence which is at least 85% similar to said product.

The invention further provides a polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 53 to 136 in SEQ ID No. 1, nucleotides 11 to 94 in SEQ ID No.2, nucleotides 24 to 107 in SEQ ID No. 3, nucleotides 20 to 103 in SEQ ID No. 4 or nucleotides 1 to 176 in SEQ ID No. 5 excluding the sequence encoding the intron marked at positions 65 to 156. These nucleotide sequences are signal sequences which may be linked to homologous and heterologous protein encoding regions to transport proteins extracellularly. The invention further extends to the use of said sequences as signal sequences. The invention further extends to the translation product of nucleotides 53 to 136 in SEQ ID No. 1, nucleotides 11 to 94 in SEQ ID No.2, nucleotides 24 to 107 in SEQ ID No. 3, nucleotides 20 to 103 in SEQ ID No. 4 or nucleotides 1 to 176 in SEQ ID No. 5 and protein having an amino acid sequence which is at least 85% similar to said product.

It is preferred that the degree of similarity is at least 90%, more preferred that the degree of similarity is at least 95% and still more preferred that the degree of similarity is at least 97%.

In the context of the present invention, two amino acid sequences with at least 85% similarity to each other have at least 85% similar (identical or conservatively replaced)

amino acid residues in a like position when aligned optimally allowing for up to 3 gaps, with the proviso that in respect of the gaps a total of not more than 15 amino acid residues is affected. Likewise, two amino acid sequences with at least 90% similarity to each other have at least 90% identical or conservatively replaced amino acid residues in a like position when aligned optimally allowing for up to 3 gaps with the proviso that in respect of the gaps a total of not more than 15 amino acid residues is affected.

For the purpose of the present invention, a conservative amino acid is defined as one which does not alter the activity/function of the protein when compared with the unmodified protein. In particular, conservative replacements may be made between amino acids within the following groups:

(i) Alanine, Serine, Glycine and Threonine

(ii) Glutamic acid and Aspartic acid

(iii) Arginine and Lysine

(iv) Isoleucine, Leucine, Valine and Methionine

(v) Phenylalanine, Tyrosine and Tryptophan

Sequence alignments to measure sequence similarity may be produced using the Lasergene program MegAlign (supplied by DNASTAR Inc. 1228 S. Park St. Madison WI 53715, USA). The Clustal Method and the PAM250 residue weight table with the following parameters may be used:

Multiple Alignment Parameters

gap penalty 10

gap length penalty 10

Pairwise alignment parameters

Ktuple 1

gap penalty 3

window 5

Diagonals saved 5

The invention also includes a polynucleotide encoding a protein having a substantially similar activity to any one of the group selected from those encoded by SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4 or SEQ ID No. 5, which polynucleotide is complementary to one which when incubated at a temperature of between 55 and 65°C in a

solution containing 5 X SSC (saline sodium citrate buffer) containing 0.1% SDS and 0.25% powdered skimmed milk followed by washing at the same temperature with 0.1, 0.5 or 2x SSC containing 0.1% SDS still hybridises with a sequence depicted in SEQ ID No 1, SEQ ID No 2, SEQ ID No3, SEQ ID No.4 or SEQ ID No.5 with the proviso that the sequence is not that described in SEQ ID No.6 or 7.

The polynucleotide sequence provided in SEQ ID Nos 6 and 7 is the predicted DNA sequence for Dm-AMP1 and Dm-AMP2 as described in Figure 31A of Published International Patent Application No. WO 93/05153.

The invention still further includes a polynucleotide encoding a protein having a substantially similar activity to any one of the group selected from that encoded by nucleotides 53 to 385 in SEQ ID No. 1, by nucleotides 11 to 334 in SEQ ID No. 2, by nucleotides 24 to 317 in SEQ ID No. 3, by nucleotides 20 to 343 in SEQ ID No. 4 or by nucleotides 1 to 446 in SEQ ID No.5 which polynucleotide is complementary to one which when incubated at a temperature of between 55 and 65°C in a solution containing 5 X SSC (saline sodium citrate buffer) containing 0.1% SDS and 0.25% powdered skimmed milk followed by washing at the same temperature with 0.1, 0.5 or 2x SSC containing 0.1% SDS still hybridises with a sequence depicted by nucleotides 53to 385 in SEQ ID No. 1, by nucleotides 11 to 334 in SEQ ID No. 2, by nucleotides 24 to 317 in SEQ ID No. 3, by nucleotides 20 to 343 in SEQ ID No. 4 or by nucleotides 1 to 446 in SEQ ID No.5. with the proviso that said sequence is not that described in SEQ ID No. 6 or SEQ ID No. 7.

The invention still further includes a polynucleotide encoding a protein having a substantially similar activity to any one of the group selected from that encoded by nucleotides 137 to 286 in SEQ ID No. 1, by nucleotides 95 to 244 in SEQ ID No. 2, by nucleotides 108 to 257 in SEQ ID No. 3, by nucleotides 104 to 253 in SEQ ID No. 4 or by nucleotides 177 to 326 in SEQ ID No.5 which polynucleotide is complementary to one which when incubated at a temperature of between 55 and 65°C in a solution containing 5 X SSC (saline sodium citrate buffer) containing 0.1% SDS and 0.25% powdered skimmed milk followed by washing at the same temperature with 0.1, 0.5 or 2x SSC containing 0.1% SDS still hybridises with a sequence depicted by nucleotides 137 to 286 in SEQ ID No. 1, by nucleotides 95 to 244 in SEQ ID No. 2, by nucleotides 108 to 257 in SEQ ID No. 3, by

nucleotides 104 to 253 in SEQ ID No. 4 or by nucleotides 177 to 326 in SEQ ID No.5. with the proviso that said sequence is not that described in SEQ ID No. 6 or SEQ ID No. 7.

The invention still further includes a polynucleotide encoding a protein having a substantially similar activity to any one of the group selected from that encoded by nucleotides 287 to 385 in SEQ ID NO. 1, nucleotides 245 to 334 in SEQ ID No. 2, nucleotides 258 to 317 in SEQ ID No. 3, nucleotides 254 to 343 in SEQ ID No. 4 or nucleotides 327 to 446 in SEQ ID No.5. which polynucleotide is complementary to one which when incubated at a temperature of between 55 and 65°C in a solution containing 5 X SSC (saline sodium citrate buffer) containing 0.1% SDS and 0.25% powdered skimmed milk followed by washing at the same temperature with 0.1, 0.5 or 2x SSC containing 0.1% SDS still hybridises with a sequence depicted by nucleotides 287 to 385 in SEQ ID NO. 1, nucleotides 245 to 334 in SEQ ID No. 2, nucleotides 258 to 317 in SEQ ID No. 3, nucleotides 254 to 343 in SEQ ID No. 4 or nucleotides 327 to 446 in SEQ ID No.5. with the proviso that said sequence is not that described in SEQ ID No. 6 or SEQ ID No. 7.

It may be desired to target the translation products of the polynucleotide to specific sub-cellular compartments within the plant cell, in which case the polynucleotide comprises sequences encoding chloroplast transit peptides, cell wall targeting sequences etc. immediately 5' of the regions encoding the said translation products.

Translational expression of the protein encoding sequences contained within the polynucleotide may be relatively enhanced by including known non translatable translational enhancing sequences 5' of the said protein encoding sequences. The skilled man is very familiar with such enhancing sequences, which include the TMV-derived sequences known as omega, and omega prime, as well as other sequences derivable, inter alia, from the regions 5' of other viral coat protein encoding sequences.

In a particularly preferred embodiment of the invention, the polynucleotide is modified in that mRNA instability motifs and/or fortuitous splice regions are removed, or plant preferred codons are used so that expression of the thus modified polynucleotide in a plant yields substantially similar protein having a substantially similar activity/function to that obtained by expression of the unmodified polynucleotide in the organism in which the protein encoding regions of the unmodified polynucleotide are endogenous, with the proviso that if the thus modified polynucleotide comprises plant preferred codons, the degree of

identity between the modified polynucleotide and a polynucleotide endogenously contained within the said plant and encoding substantially the same protein is less than about 60%.

The invention also includes a plant transformation vector comprising a plant operable promoter, a polynucleotide sequence comprising all or part of the sequence selected from those depicted in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4 and SEQ ID No.5 under the transcriptional control thereof and encoding an antimicrobial protein, and a plant operable transcription terminator. The promoter may be constitutive or inducible. In particular, the promoter may be such that it induces transcription in response to application to the plant material containing it of a chemical.

The invention further provides a plant transformation vector comprising a polynucleotide sequence selected from the group consisting of nucleotides 137 to 286 in SEQ ID No. 1, nucleotides 95 to 244 in SEQ ID No. 2, nucleotides 108 to 257 in SEQ ID No. 3, nucleotides 104 to 253 in SEQ ID No. 4 or nucleotides 177 to 326 in SEQ ID No.5 under the transcriptional control of a plant operable promoter, and a plant operable transcriptional terminator.

The polynucleotide sequences provided in SEQ ID No 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4 and SEQ ID No.5 are related sequences with the translated products thereof showing a high degree of sequence similarity and it is believed that they may belong to a multi gene family.

The invention still further includes plant tissue transformed with the said polynucleotide or vector, and material derived from the said transformed plant tissue, as well as morphologically normal fertile whole plants comprising the tissue or material. Such transformed plants include but are not limited to, field crops, fruits and vegetables such as canola, sunflower, tobacco, sugar beet, cotton, maize, wheat, barley, rice, sorghum, tomato, mango, peach, apple, pear, strawberry, banana, melon, potato, carrot, lettuce, cabbage, onion, etc. Particularly preferred genetically modified plants are bananas.

The invention still further includes the progeny of the plants of the preceding paragraph, which progeny comprises a polynucleotide of the invention stably incorporated into its genome and heritable in a mendelian manner and the seeds of such plants and such progeny.

The invention also provides a method of producing plants which are substantially tolerant or substantially resistant to antimicrobial infection, comprising the steps of:

(i) transforming plant material with a polynucleotide or vector of the invention;

(ii) selecting the thus transformed material; and

(iii) regenerating the thus selected material into morphologically normal fertile whole plants.

Plant transformation, selection and regeneration techniques, which may require routine modification in respect of a particular plant species, are well known to the skilled man.

The invention also provides the use of a polynucleotide as described herein or a vector described herein in the production of plant tissues and/or morphologically normal fertile whole plants which are substantially tolerant or substantially resistant to microbial infection.

In a further aspect the invention provides a method of selectively controlling microorganisms at a locus comprising the plants, progeny and/or seeds described herein comprising applying to the locus a microorganism controlling amount of the translation product of the region comprised by nucleotides 137 to 286 in SEQ ID No. 1.; nucleotides 95 to 244 in SEQ ID No.2, nucleotides 108 to 257 in SEQ ID No. 3, or nucleotides 104 to 253 in SEQ ID No. 4.

In a still further aspect the invention provides the use of a polynucleotide according to the invention described herein , or a vector as described herein in the production of an antimicrobial protein.

The invention will be further apparent from the following description taken in conjunction with the associated figures and sequence listings in which :

Figure 1 shows the polynucleotide and corresponding amino acid sequences for A) Dm1 (SEQ ID No5) and B) Dm2.18 (SEQ ID No1),

Figure 2 shows the polynucleotide and corresponding amino acid sequences for A) Dm2.1 (SEQ ID No2) and B) Dm2.3 (SEQ ID No3),

Figure 3 shows the polynucleotide and corresponding amino acid sequence for Dm2.5 (SEQ ID No. 4),

Figure 4 shows a diagrammatic map of plasmids pMJB1, pDmAMPD and pDmAMPE;

Figure 5 shows a diagrammatic map of plasmid pFAJ3106;

Figure 6 shows a diagrammatic map of plasmid pFAJ3109

Figure 7 shows the nucleotide sequence between the XhoI and SacI sites of pFAJ3106;

Figure 8 shows the nucleotide sequence between the XhoI and SacI sites of pFAJ3109;

Figure 9 shows a diagrammatic map of plasmid pZPS38;

Figure 10 shows a diagrammatic map of plasmid pZPS34;

Figure 11 shows a diagrammatic map of plasmid pZPS35;

Figure 12 shows a diagrammatic map of plasmid pZPS37.

Figure 13 shows a plan of the construction of the Dm-AMP gene

Figure 14 shows one predicted polynucleotide sequence for DmAMP1 (SEQ ID No. 6) and Dm-AMP2 (SEQ ID No. 7).

Figure 15 shows a diagrammatic map of plasmid pAID-MR7

EXAMPLE 1

Dm Gene Isolation and Vector Construction

Dahlia cDNA library construction

Near-dry seeds were collected from flowers of Dahlia merkii .

Total RNA was purified from the seeds using the method of Jepson et al . (Plant Molecular Biology Reporter 9 131-138 (1991)).

Seeds were frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. Phenol/m-cresol (9:1) was added followed by RNA homogenisation buffer and the mixture ground until a fine paste was obtained. The mixture was spun, the aqueous phase collected and extracted twice with phenol/chloroform (1:1). Lithium chloride (12 M) was added to the resulting aqueous layer to a final concentration of 2 M and incubated overnight at 4°C. Precipitated RNA was collected by spinning at 13,000 rpm in an Eppendorf centrifuge and the RNA pellet re-suspended in 5 mM Tris-HCl, pH 7.5. A second overnight lithium chloride

precipitation was carried out and the RNA collected and re-suspended in 5 mM Tris-HCl, pH 7.5.

0.6 mg of total RNA was obtained from 2 g of D. merkii seed.

PolyATract magnetic beads (Promega) were used to isolate approximately 2 μg poly-A^+ RNA from 0.2 mg of total RNA.

The poly-A^+ RNA was used to construct a cDNA library using a ZAP-cDNA synthesis kit (Stratagene). Following first and second strand synthesis double stranded cDNA was size fractionated on a Sephacryl S-400 column. The three largest cDNA size fractions were pooled and ligated with vector DNA. After phage assembly using Gigapack Gold (Stratagene) packaging extracts, approximately 1 x 10^5 pfu were obtained.

Probing

Dahlia genomic DNA was prepared from 100 mg of developing Dahlia seeds and flower tissue. Tissue was homogenised in a 1.5 ml Eppendorf tube with a conical plastic pestle. 400 μl of a solution containing 0.2M Tris-HCl pH 8.5, 0.25M NaCl, 0.025 M EDTA and 0.5% SDS was added and the tube vortexed for 5 seconds. Cell debris was pelleted by spinning at 13,000 rpm for 1 minute in a MSE bench top micro centrifuge. 300 μl of aqueous extract was transferred to a fresh Eppendorf tube. Genomic DNA was precipitated by the addition of 300 μl isopropyl alcohol and incubation at room temperature for 2 minutes. Genomic DNA was pelleted by spinning at 13,000 rpm for 5 minutes. The ethanol/aqueous supernatant was removed from the tube by pipette and the genomic DNA pellet allowed to air dry. Genomic DNA was then resuspended in 30 μl H_2O.

To amplify a 144 bp fragment of DNA encoding 48 amino acids of the mature Dm-AMP1 a PCR was carried out with Dahlia genomic DNA and oligonucleotides AFP-5 (based on Dm-AMP1 N-terminal amino acid sequence CEKASKTW) and AFP-3EX (based on Dm-AMP1 C-terminal amino acid sequence MCFCYFNC). Using the following conditions 94°C, 60 seconds, 48°C, 12 seconds and 72°C, 60 seconds for 35 cycles. A PCR product of approximately 150 bp was isolated from a 2% agarose gel by electroelution and ethanol precipitation. The PCR product was cloned into pBluescript by ligating blunt Bluescript vector and gel isolated PCR product together using T4DNA ligase and transforming into competent *E. coli* MC1022 cells. Transformation mixes were plated onto L-agar plates containing 100 μg/ml ampicillin and incubated at 37°C for 16 hours. Colonies were picked

and cells shaken for 16 hours in 3 ml L-broth containing 100 μg/ml ampicillin at 37°C. Plasmid DNA was prepared from colonies using a Promega Wizard mini-prep kit. The inserts of 10 transformants were sequenced using a Sequenase kit (United States Biochemical). The cloned PCR product sequences represented 3 Dm-AMP1 related genes.

PCR clone 4 contained the DNA sequence AAGACGTGGTCGGGAAACTGTGGCAATACGGG ACATTGTGACAACCAATGTAAATCATGGGAGGGTGCGGCCCATGGAGCGTGTCA TGTGCGTAATGGGAAACACATGTGTTTCTGCTACTTCAAC, encoding a portion of the observed mature Dm-AMP1 protein sequence (KTWSGNCGNTGHCD NQCKSWEGAAHGACHVRNGKHMCFCYFN).

The 144 bp PCR product mixture labelled with α^{32}-P d-CTP was used to probe Hybond N (Amersham) filter lifts made from plates containing a total of 6 x 10^4 pfu of the cDNA library. The filters were hybridised at 46°C for 18 hrs in 5 x SSC, 0.1% SDS, 0.25% skimmed milk powder. Filters were washed in 2 x SSC, 0.1% SDS at 60°C. Autoradiography was carried out at -70°C with intensifying screens. Thirty potentially positive signals were observed. 22 plaques were picked and taken through two further rounds of screening. After in vivo excision 13 clones were characterised by DNA sequencing.

Four classes of Dm-AMP related peptide were encoded by the 13 cDNA clones and the sequences of these peptides are provided in SEQ ID Nos 1- 4 in the accompanying figures. Three versions of the Dm-AMP core region were represented in the four classes. One of the classes (Dm2.5 type) contained a core region which may correspond to Dm-AMP2.

None of the cDNAs encoded a core region equivalent to the observed mature Dm-AMP1 peptide sequence.

Isolation of a Mature Dm-AMP1 Gene

Using the sequence of PCR clone 4 (above) and information from the NH_2 and COOH ends of the peptides described by cDNA sequences two pairs of oligonucleotides were designed for amplification of a gene encoding the observed mature Dm-AMP1.

A PCR was carried out with Dahlia genomic DNA and oligonucleotides MATAFP-5P (based on the codons present in Dm2.1, Dm2.3, Dm2.18 and Dm2.5 encoding the N-terminal amino acid sequence M(AV)(KN)(NR)SVAF) and MATAFP-5 (based on the mature Dm-AMP1 amino acid sequence NGKHMCF) using the following conditions; 94°C, 60 seconds, 53°C,

12 seconds and 72°C, 60 seconds for 40 cycles. A PCR product of approximately 220 bp was isolated from a 2% agarose gel by electroelution and ethanol precipitation. The PCR product was cloned into pBluescript and clones sequenced as described above. A clone containing the 5' half of a Dm-AMP1 gene was identified.

A PCR was carried out with Dahlia genomic DNA and oligonucleotides MATAFP-3 (based on the mature Dm-AMP1 amino acid sequence GACHVRN) and DM25MAT-3 (based on the last two amino acids and the 3' untranslated region of Dm2.5) using the following conditions; 94°C, 60 seconds, 53°C, 12 seconds and 72°C, 60 seconds for 40 cycles. A PCR product of approximately 170 bp was isolated from a 2% agarose gel by electroelution and ethanol precipitation. The PCR product was cloned into pBluescript and clones were sequenced as described above. A clone containing the 3' half of a Dm-AMP1 gene was identified.

The 5' and 3' sections of the mature gene were combined to assemble the sequence of the mature Dm-AMP1 gene (see Figure 1 SEQ ID No.5) which is comprised of exon 1, 64 bp encoding part of the leader peptide, 92 bp intron and exon 2 encoding the end of the leader sequence, Dm-AMP1 core and C-terminal extension.

Vector Oligonucleotide design

Four oligonucleotides were designed based on the DNA sequence of the mature Dm-AMP1 gene:

DMVEC-1 top strand priming at the 5' end of the mature DmAMP-1 gene incorporating a Nco I site at the translation start of DmAMP-1 allowing cloning into pMJB1 (see Figure 4).

DMVEC-2 bottom strand priming in the 3' end of the C-terminal extension and a Sac I site for cloning in pMJB1.

DMVEC-3 top strand priming at the 5' end of the mature DmAMP-1 gene incorporating a Nco I site at the translation start of DmAMP-1 allowing cloning into pMJB1 also encoding complete signal peptide (minus intron).

DMVEC-4 bottom strand priming in the 3' end of the core region and a Sac I site for cloning in pMJB1.

A PCR was carried out with Dahlia genomic DNA and oligonucleotides DMVEC-1 and DMVEC-2 using the following conditions; 94°C, 60 seconds, 60°C, 12 seconds and 72°C, 60 seconds for 45 cycles. A PCR product of approximately 450 bp spanning the mature Dm-

AMP1 gene was obtained, this PCR product was isolated from an agarose gel and used as a template for PCRs described in vector construction below.

Vector Construction

pDmAMPD

A PCR was carried out with the DMVEC-1 and DMVEC-2 450 bp PCR product and oligonucleotides DMVEC-1 and DMVEC-4 using the following conditions, 94°C, 48 seconds, 58°C, 12 seconds and 72°C, 90 seconds for 33 cycles. The PCR product was cut with Nco I and Sac I the 60 bp Nco I/Sac I fragment was isolated and ligated with pMJB1cut with Nco I and Sac I. The ligation mix was used to transform competent *E. coli* MC1022 cells and plasmid DNA of ampicillin resistant transformants was obtained as described above.

The identity of the fragment in one of the resulting transformants was confirmed by sequencing, the clone was termed pDmAMPA.

A PCR was carried out with the DMVEC-1 and DMVEC-2 450 bp PCR product and oligonucleotides DMVEC-3 and DMVEC-4 using the following conditions; 94°C, 48 seconds, 58°C, 12 seconds and 72°C, 90 seconds for 33 cycles. The PCR product was cut with Nco I, the resulting 150 bp Nco I fragment isolated and cloned into pDmAMPA cut with Nco I. DNA sequencing confirmed that one transformant termed pDmAMPD, contained DNA encoding Dm-AMP leader and core region.

pDmAMPE

The PCR product obtained with DMVEC-1 and DMVEC-2 was cut with Nco I and Sac I the 180 bp Nco I/Sac I fragment was isolated and cloned into pMJB1 cut with Nco I and Sac I as described above.

The identity of the fragment in one of the resulting transformants was confirmed by sequencing, the clone was termed pDmAMPB.

A PCR was carried out with the DMVEC-1 and DMVEC-2 450 bp PCR product and oligonucleotides DMVEC-3 and DMVEC-4 using the following conditions; 94°C, 48 seconds, 58°C, 12 seconds and 72°C, 90 seconds for 33 cycles. The PCR product was cut with Nco I and the resulting 150 bp Nco I fragment isolated and cloned into pDmAMPB cut

with Nco I. DNA sequencing confirmed that one transformant termed pDmAMPE, contained DNA encoding Dm-AMP leader, core and C-terminal extension.

Both pDmAMPD and pDmAMPE vector sequences contained PCR derived base substitutions with respect to Dm-AMP1 gene DNA sequence however the base changes were silent having no effect on the expected amino acid sequence.

AFP-5 (to CEKASKTW)

TG(T,C)GANAANGCN(A,T)(G,C)NAA(A,G)ACNTGG

AFP-3EX (to MCFCYFNC)

CA(A,G)TT(A,G)AANTANCANAAA(A,G)CACAT

MATAFP-5P

ATGGC(C,G)AAN(A,C)(A,G)NTC(A,G)GTTGCNTT

MATAFP-5

AAACACATGTGTTTCCCATT

MATAFP-3

AGCGTGTCATGTGCGTAAT

Dm25MAT-3

TAAAGAAACCGACCCTTTCACGG

DMVEC-1

ATCGTAGCCATGGTGAATCGGTCGGTTGCGTTCTCCGCG

DMVEC-2

AAACCGACCGAGCTCACGGATGTTCAACGTTTGGAAC

DMVEC-3

ATGCATCCATGGTGAATCGGTCGGTTGCGTTCTCCGCGTTCGTTCTGATCCTTTTC

GTGCTCGCCATCTCAGATATCGCATCCGTTAGTGGAGAACTATGCGAGAAA

DMVEC-4

AGCAAGCTTTTCGGGAGCTCAACAATTGAAGTAA

EXAMPLE 2

Constructions of plant transformation vectors

Expression cassettes containing a Dm-AMP1 open reading frame functionally linked to an enhanced 35S promoter, a TMV omega translational enhancer and a Nos 3' region are isolated as restriction fragments. pDmAMPD and pDmAMPE are both digested with the restriction endonucleases HindIII and EcoRI and the appropriate restriction fragment isolated and purified. Each fragment is ligated into a binary vector (a pBIN19 derivative named pBin19i) which has also been digested with HindIII and EcoRI. The resulting constructs, named pDmAMPLC and pDmAMPLCC, incorporate the expression cassettes from pDmAMPD and pDmAMPE respectively.

pDmAMPLC and pDmAMPLCC are subsequently introduced into Agrobacterium tumefaciens strain LBA4404 and introduced into tobacco and oil seed rape using standard plant transformation methodology.

Plants are regenerated from callus tissue resistant to the selective agent kanamycin and expression of the Dm-AMP1 product is monitored by standard Western blot or ELISA methods using antibody which had been raised against Dm-AMP1 protein. A range of expression levels are detected. The Dm-AMP1 expressed in selected transgenic is further characterised following extraction and partial purification from leaves of such lines. The product is of the predicted mass, as indicted by mass spectrometry. It is also demonstrated to retain biological activity after extraction as demonstrated by retention of antifungal activity in in-vitro (micro-titre plate) assays.

EXAMPLE 3

Constructions of plant transformation vectors for polyprotein expression

Schematic representations of the plant transformation vectors used in this work, pFAJ3106 and pFAJ3109, are shown in figures 5 and 6, respectively. The nucleotide sequences comprised between the XhoI and SacI sites of these plasmids, which encompass the regions encoding antimicrobial proteins, are presented in Figures 7 and 8. The regions comprised between the XhoI and SacI sites of plasmid pFAJ3106 (shown in Figure 7) was constructed following the two-step recombinant PCR protocol of Pont-Kindom G.A.D. (1994, Biotechniques 16, 1010-1011). Primers OWB175 (5'AGGAAGTTCATTTCATTTGG) and OWB279 (5'-GCCTTTGGCACAACTTCTGCCTCTTTCCGATGAGTTGTTCGGCTTTAAGTTTGTC); were used in a first PCR reaction with plasmid pDMAMPE (see above) as a template. The second PCR reaction was done using as a template plasmid pFRG4 (Terras F.R.G. et al., 1995,

Plant Cell 7, 573-588) and as primers a mixture of the PCR product of the first PCR reaction, primer OWB175 and primer OWB172 (5'TTAGAGCTCCTATTAACAAGGAAAGTAGC, SacI site underlined). The resulting PCR product was digested with XhoI and SacI and cloned into the expression cassette vector pMJB1 (see above). The expression cassette in the resulting plasmid, called pFAJ3099, was digested with HindIII (flanking the 5' end of the CaMV35S promoter) and EcoRI (flanking the 3' end of the nopaline synthase terminator) and cloned in the corresponding sites of the plant transformation vector pGPTVbar (Becker D. et al., 1992, Plant Mol. Biol. 20, 1195-1197) to yield plasmid pFAJ3106.

Plasmid pFAJ3109 was constructed by cloning the HindIII-EcoRI fragment of plasmid pDMAMPD (see above) into the corresponding sites of plant transformation vector pGPTVbar (see above).

Plant transformation

Arabidopsis thaliana ecotype Columbia-O was transformed using recombinant Agrobacterium tumefaciens by the inflorescence infiltration method of Bechtold N. et al. (1993, C.R. Acad. Sci. 316, 1194-1199). Transformants were selected on a sand/perlite mixture subirrigated with water containing the herbicide Basta (Agrevo) at a final concentration of 5 mg/l for the active ingredient phosphinothricin.

Elisa assays and protein assays

Antisera were raised in rabbits injected with either RsAFP2 (purified as described in Terras F.R.G. et al., 1992, J. Biol. Chem. 267, 15301-15309) or DmAMP1 (purified as in Osborn R.W. et al., 1995, FEBS Lett. 368, 257-262). ELISA assays were set up as competitive type assays essentially as described by Penninckx I.A.M.A. et al. (1996, Plant Cell 8, 2309-2323). Coating of the ELISA microtiter plates was done with 50 ng/ml RsAFP2 or DmAMP1 in coating buffer. Primary antisera were used as 1000- and 2000-fold diluted solutions (DmAMP1 and RsAFP2, respectively) in 3 % (w/v) gelatin in PBS containing 0.05 % (v/v) Tween 20.

Total protein content was determined according to Bradford (1976, Anal. Biochem. 72, 248-254) using bovine serum albumin as a standard.

Purification and characterisation of expressed proteins

Arabidopsis leaves were homogenized under liquid nitrogen and extracted with a buffer consisting of 10 mM NaH_2PO_4, 15 mM Na_2HPO_4, 100 mM KCl, 1.5 M NaCl. The

homogenate was heated for 10 min at 85°C and cooled down on ice. The heat-treated extract was centrifuged for 15 min at 15 000 x g and was injected on a reserved phase high pressure liquid chromatography column (RP-HPLC) consisting of C8 silica (0,46 cm x 25 cm; Rainin) equilibrated with 0.1 % (v/v) trifluoroacetic acid (TFA). The column was eluted at 1 ml/min in a linear gradient in 35 min. from 15 % to 50 % (v/v) acetonitrile in 0.1 % (v/v) TFA. The eluate was monitored for absorbance at 214 nm, collected as 1 ml fractions, evaporated and finally redissolved in water. The fractions were tested by ELISA assays.

Preparation of extracellular fluid and intracellular extract

Intercellular fluid was collected from Arabidopsis leaves by immersing the leaves in a beaker containing extraction buffer (10 mM NaH_2PO_4, 15 mM Na_2HPO_4, 100 mM KCl, 1.5 M NaCl). The beaker with the leaves was placed in a vacuum chamber and subjected to six consecutive rounds of vacuum for 2 min followed by abrupt release of vacuum. The infiltrated leaves were gently placed in a centrifuge tube on a grid separated from the tube bottom. The intercellular fluid was collected from the bottom after centrifugation of the tubes for 15 min at 1800 x g. The leaves were resubjected to a second round of vacuum infiltration and centrifugation and the resulting (extracellular) fluid was combined with that obtained after the first vacuum infiltration. After this step the leaves were extracted in a Phastprep (BIO101/Savant) reciprocal shaker and the extract clarified by centrifugation (10 min at 10,000 x g) and the resulting supernatant considered as the intracellular extract.

Characterization of transgenic plants and expression analysis

To explore the possibility of expressing polyprotein precursor genes in plants, three different plant transformation vectors were made with the aim to co-express two different cysteine-rich plant defensins with antifungal properties, namely RsAFP2 and DmAMP1. The polyprotein precursor regions of these constructs all featured a leader peptide region from the DmAMP1 cDNA, the mature protein domain of DmAMP1, an internal propeptide region, and the mature protein domain of RsAFP2. Construct 3106 has a propeptide consisting of a part of the DmAMP1 propeptide and a putative subtilisin-like protease processing site (IGKR) at its C-terminus.

The rationale behind construct 3106, is based on our observations that the C-terminal propeptides of DmAMP1 are cleaved off at their N-terminus when expressed as DmAMP1-preproproteins in tobacco, respectively, while this processing event does not prevent the

mature proteins from being sorted to the apoplast (De Bolle et al., 1996, Plant Mol. Biol. 31, 993-1008; R.W. Osborn and S. Attenborough, personal communication). This infers that the processing enzymes are either in the secretory pathway or in the apoplast. On the other hand, C-terminal cleavage of the internal propeptide in these constructs should be executed by a subtilisin-like protease, a member of which in yeast (Kex2) is known to occur in the Golgi apparatus (Wilcox C.A. and Fuller R.S., 1991, J. Cell. Biol. 115, 297-), while a member in tomato occurs in the apoplast (Tornero P. et al., 1997, J. Biol. Chem. 272, 14412-14419). Proteins deposited in the apoplast, the preferred deposition site for antimicrobial proteins engineered in transgenic plants (Jongedijk E. et al., 1995, Euphytica 85, 173-180; De Bolle et al., 1996, Plant Mol. Biol. 31, 993-1008) are normally synthesized via the secretory pathway, encompassing the Golgi apparatus.

Constructs were also made for expression of DmAMP1 (construct 3109, figure 6). Expression levels of DmAMP1 and RsAFP2 were analysed in leaves taken from a series of T1 transgenic Arabidopsis plants resulting from transformation with the constructs described above. Most of the tested lines transformed with the polyprotein constructs 3106 clearly expressed both DmAMP1 and RsAFP2. There was generally a good correlation between DmAMP1 and RsAFP2 levels. However, the RsAFP2 levels were generally 2 to 5-fold lower than the DmAMP1 levels. It is not known whether the apparent lower expression levels of RsAFP2 versus DmAMP1 are real or whether they result from a bias in the extraction procedure or the assays. The expression levels in the lines transformed with the polyprotein constructs 3106 were generally much higher compared to those in lines transformed with the single protein construct 3109. Hence, the use of polyprotein constructs appears to result in enhanced expression, which is an unexpected finding.

Analysis of the proteins expressed for polyprotein constructs

A transgenic line was selected among each of the populations transformed with construct 3106 and the selected lines were further bred to obtain plants homozygous for the transgenes. In order to analyse whether DmAMP1 and RsAFP2 were correctly processed in these lines, extracts from the plants were prepared as described in Materials and Methods and separated by RP-HPLC on a C8-silica column. Fractions were collected and assessed for presence of compounds cross-reacting with antibodies raised against either DmAMP1 or RsAFP2 using Elisa assays.

DmAMP1 cross-reacting compound eluted at a position identical or very close to that of authentic DmAMP1 in the line transformed with construct 3106. Likewise, a RsAFP2 cross-reacting compound was detected in the 3106 lines at an elution position identical or very close to that of authentic RsAFP2. None of the fractions reacted with both the anti-DmAMP1 and anti-RsAFP2 antibodies, indicating that an uncleaved fusion protein was not present in the extracts. No cross-reacting compounds were observed in a non-transformed line.

It is concluded that the primary translation products of the transcription units of construct 3106 (partial DmAMP1 C-terminal propeptide with subtilisin-like protease site as a linker peptide) are somehow processed to yield separate DmAMP1-cross-reacting and RsAFP2-cross-reacting portions that appear to be identical or very closely related to DmAMP1 and RsAFP2, respectively, based on their chromatographic behavior.

Analysis of the subcellular location of coexpressed plant defensins

In order to determine whether the coexpressed plant defensins are either secreted extracellularly or deposited intracellularly, extracellular fluid and intracellular extract fractions were obtained from leaves of homozygous transgenic Arabidopsis lines transformed with constructs 3106. The cytosolic enzyme glucose-6-phosphate dehydrogenase was used as a marker to detect contamination of the extracellular fluid fraction with intracellular components. As shown in Table 1, glucose-6-phosphate dehydrogenase was partitioned in a ratio of about 80/20 between intracellular extract fractions and extracellular fluid fractions. In contrast, the majority of DmAMP1 and RsAFP2 content in all transgenic plants tested was found in the extracellular fluid fractions. These results indicate that both plant defensins released from the polyprotein precursors are deposited primarily in the apoplast. Hence, all processing steps that result in cleavage of the polyprotein structure must occur either in the apoplast or along the secretory pathway.

Table 1: Relative abundance of glucose-6-phosphate dehydrogenase activity (GPD), DmAMP1 and RsAFP2 in the extracellular fluid (EF) and intracellular extract (IE) fractions obtained from transgenic Arabidopsis plants.

Construct	Relative abundance[1] (%) of					
	GPD		DmAMP1		RsAFP2	
	EF	IE	EF	IE	EF	IE
pFAJ3106	17	83	94	6	60	40

[1]Relative abundance is expressed as % of the sum of the contents in the EF and IE fractions.

EXAMPLE 4

Expression of the sweet tasting protein Brazzein in tomato

Production of transgenic tomato plants with increased accumulation of sweet tasting protein Brazzein.

Constructs were prepared containing the Dahlia (Dahlia merckii) antimicrobial protein signal peptide fused with the Brazzein gene under the transcriptional control of the Arabidopsis polyubiquitin extension protein promoter (UBQ) or the Polygalacturonase promoter (PG). Constructs were also prepared which encoded Brazzein without a signal peptide but with an N-terminal methionine by the insertion of ATG nucleotides upstream of the Brazzein gene under the expressional control of either the UBQ promoter or the PG promoter. These were prepared as follows:

Construction of the transformation vector for expression in tomato with Dahlia signal peptide fused to Brazzein under the expressional control of either the UBQ promoter or the PG promoter:

A synthetic DNA was produced which coded for the Dahlia signal peptide fused to Brazzein. The codons were optimised for expression in tomato. Using appropriate restriction sites the coding sequence was cloned into a plasmid vector. The coding region was excised from the plasmid and cloned between the promoter in question and the terminator in the correct orientation for expression.

Generation and analysis of plants transformed with the transformation vector.

The vector was transferred to Agrobacterium tumefaciens LBA4404 (a microorganism widely available to plant biotechnologists) and used to transform tomato plants. Transformation of tomato stem segments followed standard protocols (e.g. Bird et al Plant Molecular Biology 11, 651-662, 1988). Transformed plants were identified by their ability to grow on media containing the antibiotic kanamycin. Up to 30 individual plants were regenerated with each construct and grown to maturity. The presence of the construct in all of the plants was confirmed by polymerase chain reaction analysis. DNA Southern blot analysis on all plants indicated that the insert copy number was between 1 and 10. Northern blot analysis on fruit from one plant indicated that the Brazzein gene was expressed.

Brazzein production in the fruit of all plants was measured by ELISA (enzyme linked imunoabsorption assay) using a polyclonal and a monoclonal antibody raised against native Brazzein protein isolated from the fruit of the plant Pentadiplandra brazzeana Baillon Two fruit were collected from each transgenic plant at 7 days post breaker (the term breaker is used to indicate when the tomato fruit first show signs of the orange colouration characteristic of most mature tomato fruit). Total fruit protein was extracted from a sample of the pericarp of each of the fruit. The amount of Brazzein protein in the total protein extract was measured by ELISA and calculated as the amount of Brazzein per gram fresh weight of the fruit. For each plant the average Brazzein content of the two fruits was calculated. In some plants Brazzein could not be detected in the fruit using the ELISA technique. Western blot analysis of the total protein extract from some of the fruit revealed a 6.5kD protein band. which matches the predicted size of the mature Brazzein protein. This confirmed that the fruit contained Brazzein and that the signal peptide had been cleaved as if the signal peptide had not been cleaved, one would expect the protein to be larger. The Brazzein in fruit from plants which had been transformed with a construct lacking a signal peptide was not detected by Western blot. This is because the Brazzein content in these fruit is below the level of detection by western blot. ELISA is a more sensitive technique than western blot and protein was detected in these fruit by this method.

The results are summarised in Table 2 below.

TABLE 2

Construct Name	Promoter	Signal Peptide	No. of Plants Tested	Plants expressing Brazzein
pZPS34	UBQ	None	29	18
pZPS35	UBQ	Dahlia AMP1	25	23
pZPS37	PG	None	15	7
pZPS38	PG	Dahlia AMP1	13	11

Table 2 (continued)

Construct Name	Max Brazzein ng/g Fresh wt	Min Brazzein ng/g Fresh wt	Mean Brazzein in those plants expressing the gene
pZPS34	25.57	Not Detected	6.85
pZPS35	226.53	Not Detected	43.89
pZPS37	12.77	Not Detected	3.32
pZPS38	51745.77	Not Detected	12867.34

Example 5

Construction of Dm-AMP Transient Expression Vectors

To produce proteins for assessment of the relative activity of the variants of Dm-AMP, three vectors were constructed for transformation of black Mexican sweet (BMS) maize cell suspensions to give transient expression of Dm-AMPs.

The vector chosen for these experiments was pAID-MR7.

pAID-MR7 was constructed using the commercially available cloning vector pNEB193 (New England Biochemicals) itself a modified version of pUC19 (Yanisch-Perron C., Vieira J. and Messing J. "Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors." Gene; **33**:103-19 (1985)). Gene components to facilitate protein expression were inserted within the multiple cloning region of pNEB193, namely:

1) A plant promoter to drive transcription, a 1.9Kb Xba I fragment of the MR7 promoter (MR7 prom.) from maize (as described in US Patent No. 5837848)
2) A sequence known to enhance gene transcription, the alcohol dehydrogenase intron 1 (I 1) from maize (Dennis E.S., Gerlach W.L., Pryor A.J., Bennetzen J.L., Inglis A., Llewellyn D., Sachs M.M., Ferl R.J. and Peacock W.J. "Molecular analysis of the alcohol dehydrogenase (Adh1) gene of maize." Nucleic Acids Research; **12**:3983-4000(1984)).

3) A multiple cloning region for insertion of open reading frames containing the site for restriction endonuclease Xba I.

4) A 3' region for mRNA transcript termination and polyadenylation from the cauliflower mosaic virus 35S RNA transcript (3') (Pietrak et al Nucleic Acid Research 14 5857-5868 (1986), Franck A., Guilley H., Jonard G., Richards K. and Hirth L. "Nucleotide sequence of cauliflower mosaic virus DNA." Cell; **21**: 285-94 (1980)).

To produce proteins for assessment of the relative activity of the variants of Dm-AMP, three vectors were constructed for use in a transient expression system.

All plasmid DNA described in the following examples was prepared using Promega Wizard mini-prep or Promega Wizard midi-prep kits using the manufacturer's suggested protocols. DNA sequencing was carried out using USB Sequenase kits and the manufacturer's suggested protocols.

Example 5a

Vector DNA was prepared by digesting plasmid DNA of pAIDMR7 with Xba I and the ends filled in with Klenow DNA polymerase. The linear vector was isolated from an agarose gel by electroelution and ethanol precipitation. The DNA pellet was air dried and taken up in a small volume of water.

Plasmid DNA of a cDNA clone containing a Dm2.1 ORF was digested with Eco RI and Sca I and the ends filled in with Klenow DNA polymerase. Insert DNA containing the Dm2.1 coding region was isolated from an agarose gel by electroelution and ethanol precipitation. The DNA pellet was air dried and taken up in a small volume of water.

Vector and insert DNA were ligated together using T4 DNA ligase and transformed into competent *E. coli* MC1022 cells. Tranformation mixes were plated onto L-agar plates containing 100 µg/ml ampicillin and incubated at 37°C for 16 hours. Colonies were picked and cells shaken for 16 hours in 3 ml L-broth containing 100 µg/ml ampicillin at 37°C. Plasmid DNA was prepared from several colonies and used in DNA sequencing reactions to identify transformants containing the Dm2.1 coding region in the appropriate orientation with respect to the MR7 promoter.

One such clone was identified and named pAIDMR721.

Example 5b

Vector DNA was prepared as in Example 5a.

Plasmid DNA of a cDNA clone containing a Dm2.3 ORF was digested with Eco RI and Sca I and the ends filled in with Klenow DNA polymerase. Insert DNA containing the Dm2.3 coding region was isolated from an agarose gel by electroelution and ethanol precipitation. The DNA pellet was air dried and taken up in a small volume of water.

Vector and insert DNA were ligated together, transformed into *E. coli* MC1022 and colonies characterised by DNA sequencing as described in Example 5a. A clone was identified containing the Dm2.3 ORF in the desired orientation and named pAIDMR723.

Example 5c

Vector DNA was prepared as in Example 5a.

Plasmid DNA of a cDNA clone containing a Dm2.5 ORF was digested with Eco RI and Dra I and the ends filled in with Klenow DNA polymerase. Insert DNA containing the Dm2.5 coding region was isolated from an agarose gel by electroelution and ethanol precipitation. The DNA pellet was air dried and taken up in a small volume of water.

Vector and insert DNA were ligated together, transformed into *E. coli* MC1022 and colonies characterised by DNA sequencing as described in Example 5a. A clone was identified containing the Dm2.5 ORF in the desired orientation and named pAIDMR725.

Example 6

Transient Expression of Dm-AMPs

Plasmid DNA of clones pAIDMR721 pAIDMR723, pAIDMR725 and pAIDMR7 is used to transform cultured maize BMS cells using the PEG method.

Protoplast preparation and transformation

Protoplasts are isolated from a maize suspension of Black Mexican Sweet Corn suspension culture (BMS) [Green, Hort. Sci., 12 (1977) 131; Smith et al., Plant Sci. Lett., 36 (1984) 67] subcultured in BMS medium (MS medium supplemented with 2% sucrose, 2 mg/l 2,4-D, pH5.8). Cells from suspensions two days post subculture are digested in enzyme mixture (2.0% cellulase RS (Yakult Honsha Co., Ltd), 0.2% pectolyase Y23 (Yakult Honsha Co., Ltd), 0.5M mannitol, 5mM $CaCl_2 2H_2O$, 0.5% MES, pH5.6, ~660mmol/kg) using ~10ml/g

cells, incubating at 25°C, rotating gently for 2 hours. The digestion mixture is sieved through 250μm and 38μm sieves, and the filtrate centrifuged at 700rpm for 3.5 minutes. Protoplasts are resuspended in wash buffer (0.358M KCl, 1.0mM NH_4NO_3, 5.0mM $CaCl_2 2H_2O$, 0.5mM KH_2PO_4, pH4.8, ~670mmol/kg) and pelleted twice prior to resuspending in wash buffer and counting. Transformation is achieved using PEG (PEG 3350, Sigma Co) mediated uptake (Negrutiu et al., 1987) employing plasmid DNA prepared using Qiagen midi plasmid preparation kit (Qiagen Ltd, Crawley, UK). Protoplasts are resuspended at 2 x 10^6/ml in MaMg medium (0.4M mannitol, 15mM $MgCl_2$, 0.1% MES, pH5.6, ~450mmol/kg) aliquotting 0.5ml / treatment (i.e. 1x10^6 protoplasts/treatment). Samples are heat shocked at 45°C for 5 minutes then cooled to room temperature. Each transformation is carried out with 10μg of pAID-MR7 alone or 10μg of each construct pAIDMR721, pAIDMR723 or pAIDMR725. Each protoplast treatment is resuspended in 1.5ml culture medium (MS medium, 2% sucrose, 2mg/l 2,4-D, 9% mannitol, pH5.6, ~700mmol/kg). Samples are incubated in 3cm dishes at 25°C, in the dark, for 48 hours prior to harvesting.

After 48 hours incubation cells are separated from media by centrifugation.
Cells are osmotically lysed by the addition of water. Cell debris is pelleted by centrifugation and the proteins remaining in solution are freeze dried. The freeze dried proteins are taken up in a small volume of water and the concentration of protein determined using Bradford reagent.
Culture media removed from cells is freeze dried and taken up in a small volume of water, the concentration of protein is determined as above.

Protein samples isolated from all BMS transformations are assayed for spore germination inhibition in a bioassay against *Fusarium culmorum* spores as described in Published International Patent Application No. WO 93/05153.

CLAIMS

1. A polynucleotide comprising a sequence selected from those depicted in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4 and SEQ ID No. 5.

2. A polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 53 to 385 in SEQ ID No. 1, nucleotides 11 to 334 in SEQ ID No. 2, nucleotides 24 to 317 in SEQ ID No. 3, nucleotides 20 to 343 in SEQ ID No. 4 or nucleotides 1 to 446 in SEQ ID No. 5.

3. A polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 137 to 286 in SEQ ID No. 1, nucleotides 95 to 244 in SEQ ID No. 2, nucleotides 108 to 257 in SEQ ID No. 3, nucleotides 104 to 253 in SEQ ID No. 4 or nucleotides 177 to 326 in SEQ ID No. 5.

4. A polynucleotide sequence comprising a sequence selected from the group consisting of nucleotides 287 to 385 in SEQ ID NO. 1, nucleotides 245 to 334 in SEQ ID No. 2, nucleotides 258 to 317 in SEQ ID No. 3, nucleotides 254 to 343 in SEQ ID No. 4 or nucleotides 327 to 446 in SEQ ID No.5.

5. A polynucleotide encoding a protein having a substantially similar activity to that encoded by any of SEQ ID No. 1, No 2, No. 3, No,4 or No. 5 which polynucleotide is complementary to one which when incubated at a temperature of between 55 and 65°C in a solution containing 5 X SSC (saline sodium citrate buffer) containing 0.1% SDS and 0.25% powdered skimmed milk followed by washing at the same temperature with 0.1, 0.5 or 2x SSC containing 0.1% SDS still hybridises with a sequence depicted in SEQ ID No 1, SEQ ID No 2, SEQ ID No3, SEQ ID No.4 or SEQ ID No.5 with the proviso that the sequence is not that described in SEQ ID No.6 or 7.

6. A polynucleotide encoding a protein having a substantially similar activity to that encoded by nucleotides 137 to 286 in SEQ ID No. 1, nucleotides 95 to 244 in SEQ ID No. 2, nucleotides 108 to 257 in SEQ ID No. 3, nucleotides 104 to 253 in SEQ ID No. 4 or nucleotides 177 to 326 in SEQ ID No. 5., which polynucleotide is complementary to one which when incubated at a temperature of between 55 and 65°C in a solution containing 5 X SSC (saline sodium citrate buffer) containing 0.1% SDS and 0.25% powdered skimmed milk followed by washing at the same temperature with 0.1, 0.5 or 2x SSC containing 0.1% SDS still hybridises with a sequence depicted by nucleotides 137 to 286 in SEQ ID No. 1, by nucleotides 95 to 244 in SEQ ID No. 2, by nucleotides 108 to 257 in SEQ ID No. 3, by nucleotides 104 to 253 in SEQ ID No. 4 or by nucleotides 177 to 326 in SEQ ID No.5. with the proviso that said sequence is not that described in SEQ ID No. 6 or SEQ ID No. 7.

7. A polynucleotide according to any preceding claim, further comprising a region encoding a peptide which is capable of targeting the translation products of the sequence to plastids such as chloroplasts, mitochondria, other organelles or plant cell walls.

8. A polynucleotide according to any preceding claim, wherein translational enhancing sequences are inserted 5' of the protein encoding regions comprised by the polynucleotide.

9. A polynucleotide according to any preceding claim, which is modified in that mRNA instability motifs and/or fortuitous splice regions are removed, or plant preferred codons are used so that expression of the thus modified polynucleotide in a plant yields substantially similar protein having a substantially similar activity/function to that obtained by expression of the unmodified polynucleotide in the organism in which the protein encoding regions of the unmodified polynucleotide are endogenous, with the proviso that if the thus modified polynucleotide comprises plant preferred codons, the degree of identity between the modified polynucleotide and a

polynucleotide endogenously contained within the said plant and encoding substantially the same protein is less than about 60%.

10. A plant transformation vector comprising a plant operable promoter, a polynucleotide sequence according to any of the preceding claims under the transcriptional control thereof and a plant transcription terminator.

11. Plant tissue transformed with the polynucleotide of any one of claims 1 to 9 or the vector of claim 10 and material derived from the said transformed plant tissue.

12. Morphologically normal fertile whole plants comprising the tissue or material of the preceding claim.

13. The progeny of the plants of the preceding claim, which progeny comprises the polynucleotide of any one of claims 1 to 9 stably incorporated into its genome and heritable in a mendelian manner, the seeds of such plants and such progeny.

14. A method of producing plants which are substantially tolerant or substantially resistant to microbial infection, comprising the steps of:

(i) transforming plant material with the polynucleotide of any one of claims 1 to 9 or the vector of claim 10

(ii) selecting the thus transformed material; and

(iii) regenerating the thus selected material into morphologically normal fertile whole plants.

15. Use of the polynucleotide of any one of claims 1 to 9, or the vector of claim 10 in the production of plant tissues and/or morphologically normal fertile whole plants which are substantially tolerant or substantially resistant to microbial infection.

16. The translation product of the region comprised by nucleotides 137 to 286 in SEQ ID No. 1.; nucleotides 95 to 244 in SEQ ID No.2, nucleotides 108 to 257 in SEQ ID No.

3, or nucleotides 104 to 253 in SEQ ID No. 4 and protein having an amino acid sequence which is at least 95% similar to said product.

17. The translation product of the region comprised by nucleotides 287 to 385 in SEQ ID NO. 1, nucleotides 245 to 334 in SEQ ID No. 2, nucleotides 258 to 317 in SEQ ID No. 3, nucleotides 254 to 343 in SEQ ID No. 4 or nucleotides 327 to 446 in SEQ ID No.5 and protein having an amino acid sequence which is at least 85% similar to said product.

18. A method of selectively controlling microorganisms at a locus comprising the plants, progeny and/or seeds of either of claims 12 or 13, comprising applying to the locus a microorganism controlling amount of the translation product of the region comprised by nucleotides 137 to 286 in SEQ ID No. 1.; nucleotides 95 to 244 in SEQ ID No.2, nucleotides 108 to 257 in SEQ ID No. 3, or nucleotides 104 to 253 in SEQ ID No. 4.

19. Use of the polynucleotide of any one of claims 1 to 9, or the vector of claim 10 in the production of an antimicrobial protein.

Dm Gene Structure Sequence ID No. 5 Fig.1A.

```
      M  V  N  R  S  V  A  F  S  A  F  V  L  I  L  F  V  L  A  I
1     ATGGTGAATCGGTCGGTTGCGTTCTCCGCGTTCGTTCTGATCCTTTTCGTGCTCGCCATC

      S
61    TCAGGTTATCAAATCTTTAGTTCATTTATTGAATATGATAGTATTTATATTCTTTTATGG
                                                  intron
                                                  D  I  A  S  V  S  G  E
121   TTTTATGTGTTCTGACAAGTTGCAAATATTGAGTAGATATCGCATCCGTTAGTGGAGAAC

      L  C  E  K  A  S  K  T  W  S  G  N  C  G  N  T  G  H  C  D
181   TATGCGAGAAAGCTAGCAAGACATGGTCGGGAAACTGTGGCAATACGGGACATTGTGACA
                                     NcoI
      N  Q  C  K  S  W  E  G  A  A  H  G  A  C  H  V  R  N  G  K
241   ACCAATGTAAATCATGGGAGGGTGCGGCCCATGGAGCGTGTCATGTGCGTAACGGGAAAC
                                                 HindIII
      H  M  C  F  C  Y  F  N  C  K  K  A  E  K  L  A  Q  D  K  L
301   ACATGTGTTTCTGTTACTTCAATTGTAAAAAAGCCGAAAAGCTTGCTCAAGACAAACTTA
                                                 HindIII
      K  A  E  Q  L  A  Q  D  K  L  N  A  Q  K  L  D  R  D  A  K
361   AAGCCGAACAACTCGCTCAAGACAAACTTAATGCCCAAAAGCTTGACCGTGATGCCAAGA

      K  V  V  P  N  V  E  H  P
421   AAGTGGTTCCAAACGTTGAACATCCG
```

Dm2.18 Sequence ID No. 1 Fig.1B.

```
                                                             M  A  K
  1  GTGCCCCGGGTCACGAAGTTCGGCACATCTTAGCGTTATGCATAAGTCAAAAATGGCCAA

      N  S  V  A  F  F  A  L  C  L  L  L  F  I  L  A  I  S  E  I
 61  AAATTCAGTTGCTTTCTTTGCATTGTGCCTGCTTCTTTTCATTCTTGCTATCTCAGAAAT

      R  S  V  K  G  E  L  C  E  K  A  S  K  T  W  S  G  N  C  G
121  CAGATCGGTGAAGGGGGAATTATGTGAGAAGGCAAGCAAGACATGGTCTGGAAATTGTGG

      N  T  R  H  C  D  D  Q  C  K  S  W  E  G  A  A  H  G  A  C
181  CAATACAAGACACTGTGATGACCAGTGCAAGTCTTGGGAGGGTGCAGCCCATGGAGCTTG

      H  V  R  G  G  K  H  M  C  F  C  Y  F  N  C  P  K  A  Q  K
241  TCACGTGCGCGGTGGGAAACACATGTGCTTCTGCTACTTCAACTGTCCCAAAGCCCAGAA

      L  A  E  D  K  L  R  A  A  E  L  A  K  E  K  N  N  I  G  A
301  GTTGGCTGAGGATAAACTCAGAGCAGCAGAGCTAGCAAAGGAGAAGAATAATATTGGAGC

      E  K  V  P  S  A  T  P
361  TGAAAAGGTGCCTTCAGCCACACCTTGAGTACTAACAAA
```

Dm 2.1 **Sequence ID No. 2** Fig.2A.

```
                M  A  K  N  S  V  A  F  L  A  F  L  L  L  L  F  V
  1 GGCACGAGTAATGGCCAAAAATTCAGTTGCTTTCTTAGCATTTCTTCTGCTTCTTTTCGT

                L  A  I  S  E  I  G  S  V  K  G  E  L  C  E  K  A  S  K  T
 61 TCTTGCTATCTCAGAAATCGGATCGGTGAAGGGGGAATTATGTGAGAAGGCAAGCAAGAC

                W  S  G  N  C  G  N  T  R  H  C  D  D  Q  C  K  S  W  E  G
121 ATGGTCTGGAAATTGTGGCAATACAAGACACTGTGATGACCAGTGCAAGTCTTGGGAGGG

                A  A  H  G  A  C  H  V  R  G  G  K  H  M  C  F  C  Y  F  N
181 CGCAGCCCATGGAGCTTGTCACGTGCGCGGTGGGAAACACATGTGCTTTTGCTACTTCAA

                C  S  K  A  Q  K  L  A  Q  D  K  L  K  A  D  K  L  A  K  E
241 CTGTTCCAAAGCCCAGAAGCTGGCTCAGGATAAACTCAAAGCCGACAAGCTCGCCAAGGA

                K  S  E  A  E  K  V  P  A  T  P
301 GAAGAGTGAAGCCGAAAAGGTGCCAGCTACACCTTGAGTACTAACAAGTGTTGTATGATT

361 ATGAATAAAGAGAAAATGCTTTCTAGTTACCATATTTAGCATTCTCTAATGTGTAATGTT

421 TGTTGCTTTTGGAACTAATTGCTTAACTATGATTCCAGCTAATAATGTTTTAAGTATATA

481 ATATAAGTTATCTTATTTTGAAGCCTGTAAAAAAAAAAAAAAA
```

Dm2.3 Sequence ID No. 3 Fig.2B.

```
                M  A  K  N  S  V  A  F  F  A  F  V
1   CGGCACGAGGCACAATCTCAAAAATGGCCAAAAATTCGGTTGCTTTCTTTGCATTTGTCC

                L  L  L  F  V  L  A  I  S  E  I  G  S  V  K  G  E  L  C  E
61  TGCTTCTTTTCGTTCTTGCTATCTCAGAATTGGATCGGTGAAGGGAGAATTATGTGAGA

                K  A  S  K  T  W  S  G  N  C  G  I  T  S  H  C  D  N  Q  C
121 AGGCAAGCAAGACATGGTCTGGAAATTGTGGCATCACATCACACTGTGACAACCAGTGCC

                R  S  W  E  G  A  I  H  G  A  C  H  V  R  G  G  K  H  M  C
181 GGTCGTGGGAGGGTGCAATCCATGGAGCTTGTCACGTGCGCGGTGGGAAACACATGTGCT

                F  C  Y  F  N  C  S  K  A  D  E  L  A  K  E  K  I  E  A  E
241 TCTGCTACTTCAACTGTTCCAAGCCGATGAGCTCGCGAAGGAGAAGATTGAAGCCGAAA

                K  M  P  A  T  P
301 AGATGCCAGCCACACCTTGAGTACTAACAAATGCTATATGATTATAAATAAAGAGAAAAT

361 GCTTTCTAAAAAAAAAAAAAAAAA
```

Dm2.5 Sequence ID No. 4 Fig.3.

```
                M  V  N  N  R  S  V  A  F  S  V  F  V  L  I
1   GGCACGAGCCTATTAAAAATGGTGAATCGATCGGTTGCTTTCTCCGTGTTCGTTCTGAT

                L  F  V  L  A  I  S  D  I  T  S  V  R  G  E  V  C  E  K  A
61  CCTTTCGTGCTCGCCATCTCAGATATCACAAGTGTGAGAGGAGAAGTATGCGAGAAAGC

                S  K  T  W  S  G  N  C  G  N  T  G  H  C  D  N  Q  C  K  Y
121 TAGCAAGACATGGTCAGGAAACTGTGGCAACACGGGACACTGTGACAACCAATGTAAATA

                W  E  G  A  A  H  G  A  C  H  V  R  G  G  G  K  H  M  C  F  C
181 CTGGGAGGGGGCGGCCCATGGGGCGTGCCACGTGCGTGGAGGGAAACACATGTGTTCTG

                Y  F  K  C  P  K  A  E  K  L  A  Q  D  K  V  N  A  Q  E  L
241 CTACTTCAAGTGTCCCAAAGCCGAAAAGCTTGCTCAAGACAAAGTTAATGCCCAAGAGCT

                D  R  D  A  K  K  V  I  P  N  V  E  H  P
301 TGACCGTGATGCCAAGAAAGTGATTCCGAACGTTGAACATCCGTGAAAGGGTCGGTTTCT

361 TTAAATAGAAAGTCTTAGATTACGAATGCGAATAACTATAGAAAATGTTTGCTAAATGTC

421 ACATTATAATTAGAACTTTATGATTGTTGTCAATAGGGCATTTTCTTGTTAGTGATATGT

481 GTAATAAGGTGATGCTTTTATGCTTTTCGTGCGTAAGAGTTTTCGACTATGTGTAATAAA

541 GAAAGGGTCTTTTTTTTTAAAAAAAAAAAAAAAA
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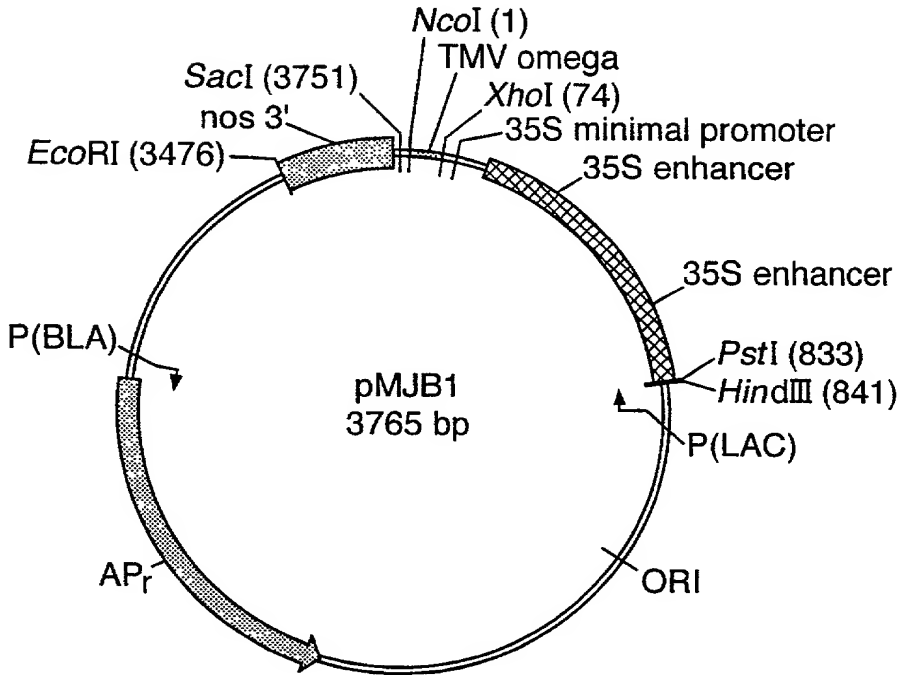
Fig.4.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TMV omega
SacI (3751)
XhoI (74)
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35S minimal promoter
EcoRI (3476)
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P(BLA)
PstI (833)
HindIII (841)
pMJB1
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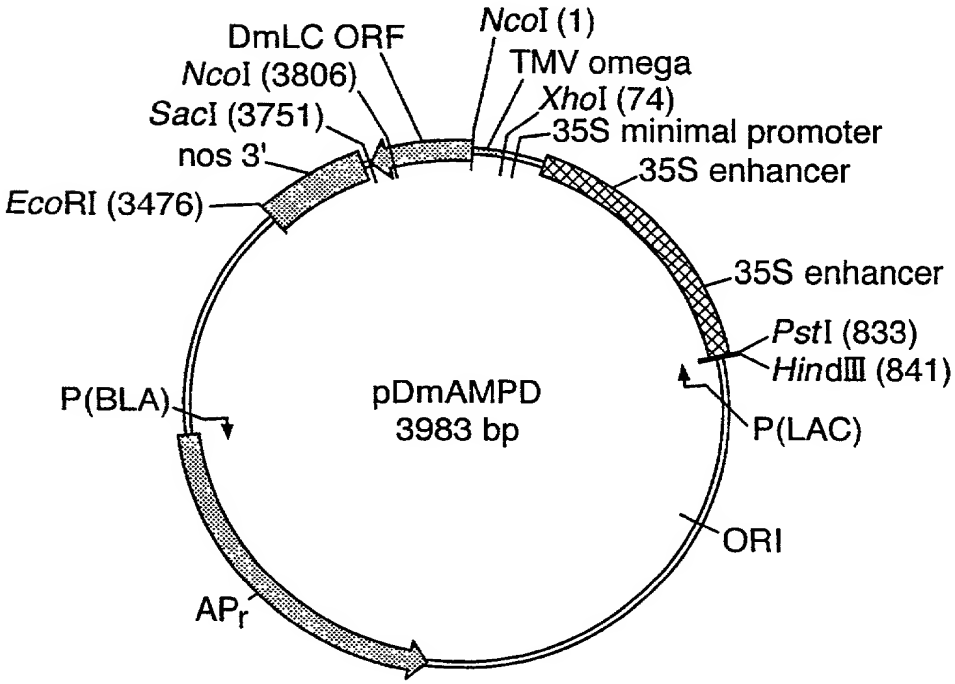
P(LAC)
APr
ORI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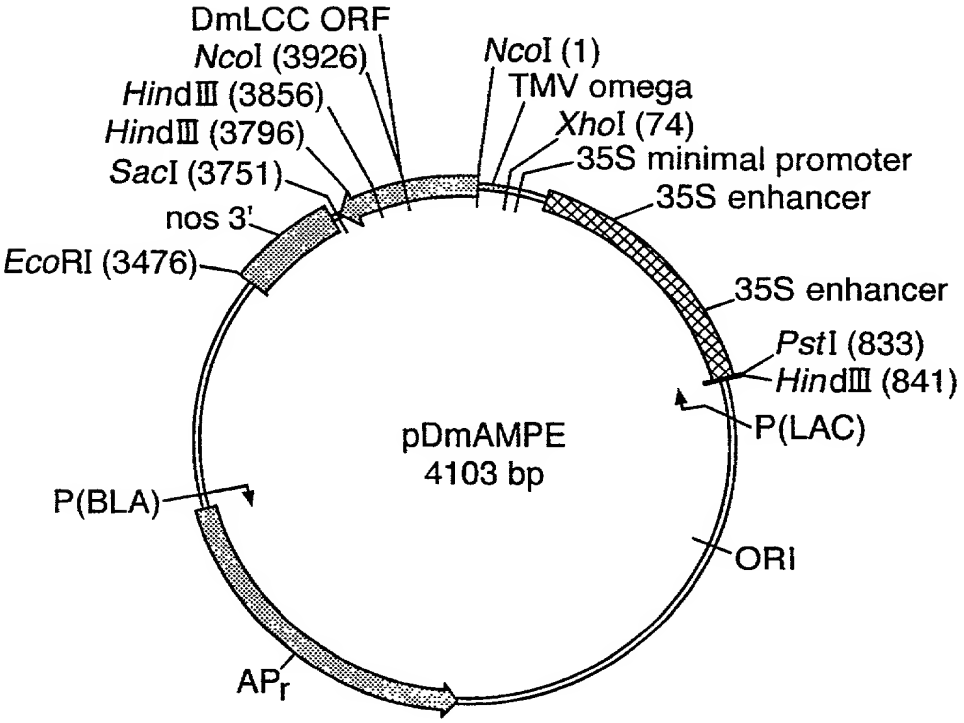
NcoI (3806)
TMV omega
SacI (3751)
XhoI (74)
nos 3'
35S minimal promoter
EcoRI (3476)
35S enhancer
35S enhancer
PstI (833)
HindIII (841)
P(BLA)
pDmAMPD
3983 bp
P(LAC)
ORI
APr

Fig.4 (Cont).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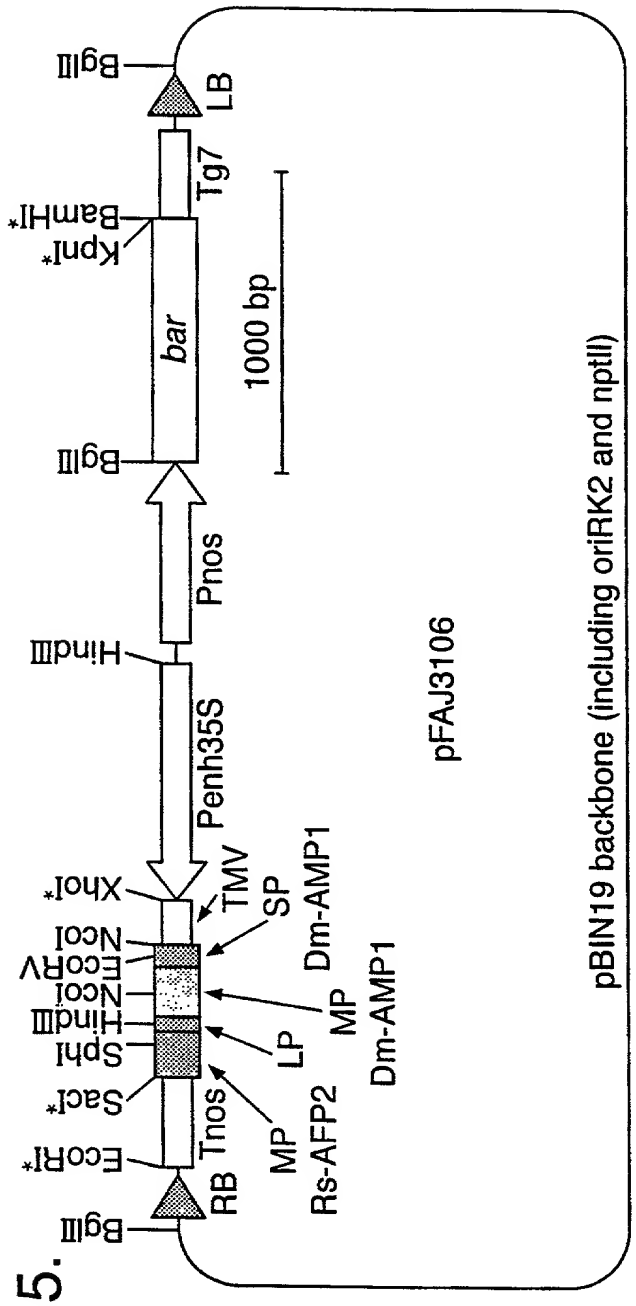
HindIII (3796)
SacI (3751)
nos 3'
EcoRI (3476)
NcoI (1)
TMV omega
XhoI (74)
35S minimal promoter
35S enhancer
35S enhancer
PstI (833)
HindIII (841)
P(LAC)
pDmAMPE
4103 bp
P(BLA)
ORI
APr

Fig.5.



Symbols

RB: right border of T-DNA
Tnos: terminator of T-DNA nopaline synthase gene
MP Rs-AFP2: mature protein domain of Rs-AFP2
LP: first 16 AA of Dm-AMP1 C-terminal propeptide and subtilisin-like protease recognition site IGKR
MP Dm-AMP1: mature protein domain of Dm-AMP1 cDNA
SP Dm-AMP1: signal peptide domain of Dm-AMP1 cDNA
TMV: tobacco mosaic virus 5' leader sequence
Penh35S: promotor of 35S RNA of cauliflower mosaic virus with duplicated enhancer region
Pnos: promotor of T-DNA nopaline synthase gene
bar: basta resistance encoding gene
Tg7: terminator of T-DNA gene 7
LB: left border of T-DNA

*: unique restriction site

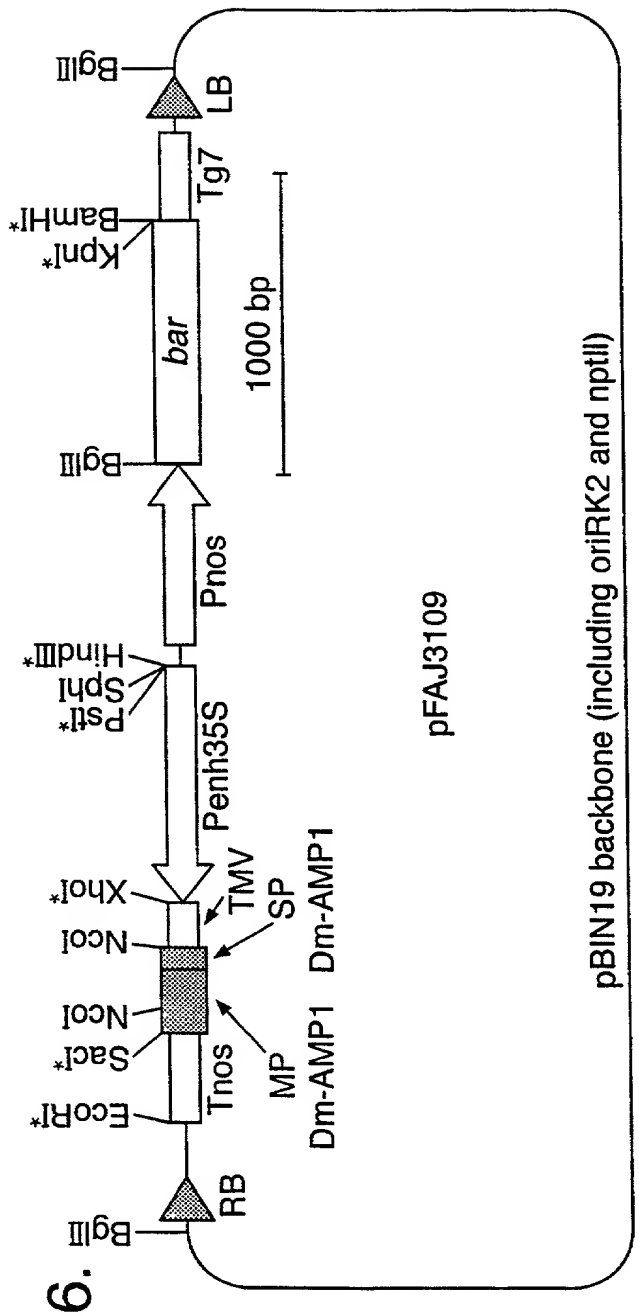


Fig.6.

Symbols

RB: right border of T-DNA
Tnos: terminator of T-DNA nopaline synthase gene
MP Dm-AMP1: mature protein domain of Dm-AMP1
SP Dm-AMP1: signal peptide domain of Dm-AMP1 cDNA
TMV: tobacco mosaic virus 5' leader sequence
Penh35S: promotor of 35S RNA of cauliflower mosaic virus with duplicated enhancer region
Pnos: promotor of T-DNA nopaline synthase gene
bar: basta resistance encoding gene
Tg7: terminator of T-DNA gene 7
LB: left border of T-DNA

*: unique restriction site

Fig.7.

pFAJ3106

XhoI
CTCGAGTATTTTTACAACAATTACCAACAACAACAAACAACAACATTACAATTACT

NcoI
ATTTACAATTACACCATGGTGAATCGGTCGGTTGCGTTCTCCGCGTTCGTTCTGATCCTT
M V N R S V A F S A F V L I L

TTCGTGCTCGCCATCTCAGATATCGCATCCGTTAGTGGAGAACTATGCGAGAAAGCTAGC
F V L A I S D I A S V S G E L C E K A S

AAGACGTGGTCGGGCAACTGTGGCAACACGGGACATTGTGACAACCAATGTAAATCATGG
K T W S G G N C G N T G H C D N Q C K S W

GAGGGTGCGGCCCATGGAGCGTGTCATGTGCGTAACGGGAAACACATGTGTTTCTGTTAC
E G A A H G G A C H V R N G K H M C F C Y

TTCAATTGTAAAAAAGCCGAAAAGCTTGCTCAAGACAAACTTAAAGCCGAACAACTCATC
F N C K K A E K L A Q D K L K A E Q L I

GGAAAGAGGCAGAAGTTGTGCCAAAGGCCAAGTGGGACATGGTCAGGAGTCTGTGGAAAC
G K R Q K L C Q R P S G T W S G V C G N

AATAACGCATGCAAGAATCAGTGCATTAGACTTGAGAAAGCACGACATGGATCTTGCAAC
N N A C K N Q C I R L E K A R H G S C N

SacI
TATGTCTTCCCAGCTCACAAGTGTATCTGCTACTTTCCTTGTTAATAGGAGCTC
Y V F P A H K C I C Y F P C - -

Fig.8.

pFAJ3109

XhoI
CTCGAGTATTTTTACAACAATTACCAACAACAACAAACAACAAACAACATTACAATTACT

NcoI
ATTTACAATTACACCATGGTGAATCGGTCGGTTGCGTTCTCCGCGTTCGTTCTGATCCTT
M V N R S V A A F S A F V L I L

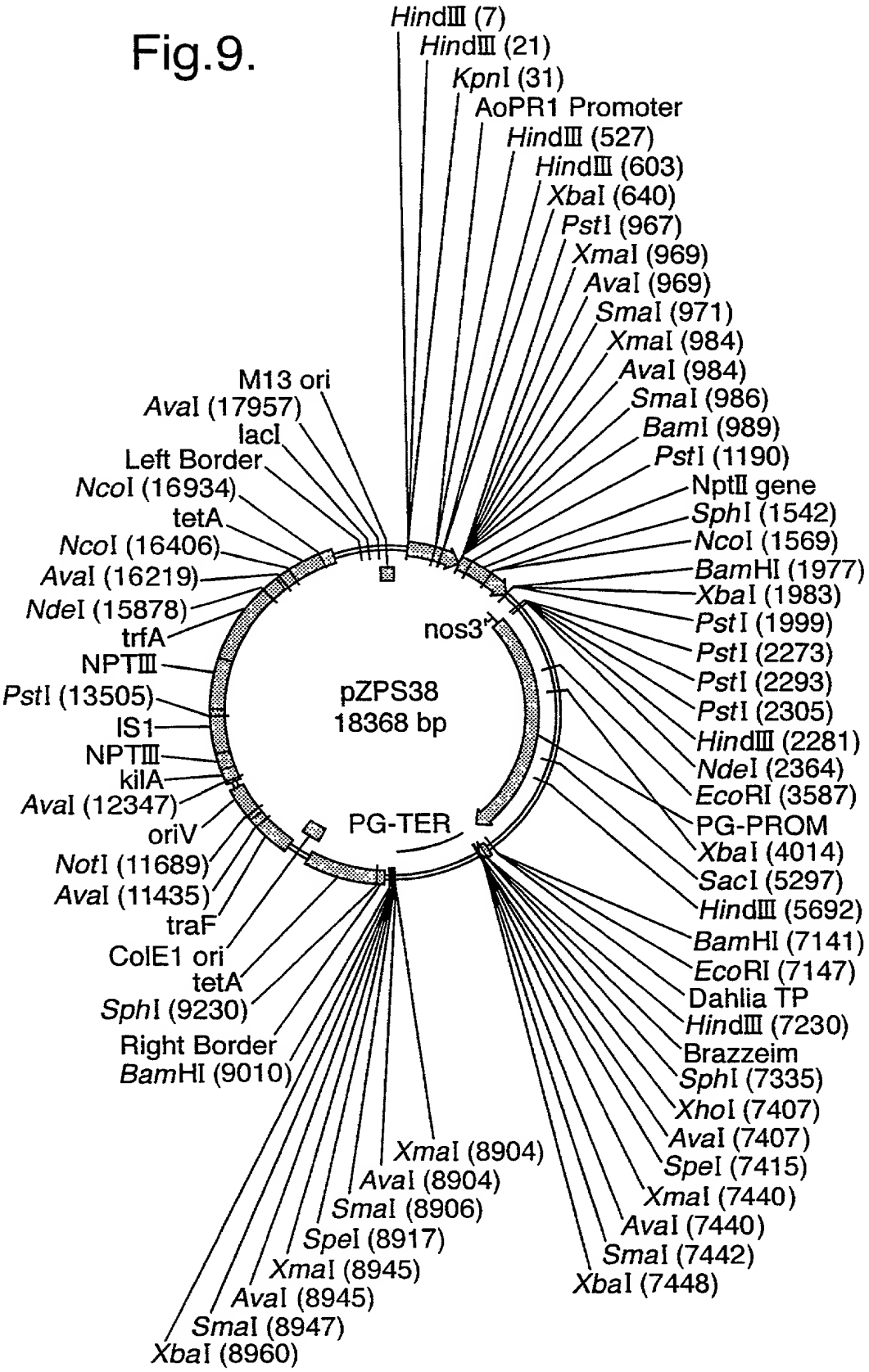
TTCGTGCTCGCCATCTCAGATATCGCATCCGTTAGTGGAGAACTATGCGAGAAAGCTAGC
F V L A I S D I A S V S G E L C E K A S

AAGACGTGGTCGGGCAACTGTGGCAACACGGGACATTGTGACAACCAATGTAAATCATGG
K T W S G N C G N T G H C D N Q C K S W

GAGGGTGCGGCCCATGGAGCGTGTCATGTGCGTAATGGGAAACACATGTGTTTCTGTTAC
E G A A H G A C H V R N G K H M C F C Y

SacI
TTCAATTGTTGAGCTC
F N C -

Fig.9.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HindIII (21)
KpnI (31)
AoPR1 Promoter
HindIII (527)
HindIII (603)
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PstI (967)
XmaI (969)
AvaI (969)
SmaI (971)
XmaI (984)
AvaI (984)
SmaI (986)
BamI (989)
PstI (1190)
NptII gene
SphI (1542)
NcoI (1569)
BamHI (1977)
XbaI (1983)
PstI (1999)
PstI (2273)
PstI (2293)
PstI (2305)
HindIII (2281)
NdeI (2364)
EcoRI (3587)
PG-PROM
XbaI (4014)
SacI (5297)
HindIII (5692)
BamHI (7141)
EcoRI (7147)
Dahlia TP
HindIII (7230)
Brazzeim
SphI (7335)
XhoI (7407)
AvaI (7407)
SpeI (7415)
XmaI (7440)
AvaI (7440)
SmaI (7442)
XbaI (7448)
XmaI (8904)
AvaI (8904)
SmaI (8906)
SpeI (8917)
XmaI (8945)
AvaI (8945)
SmaI (8947)
XbaI (8960)
Right Border
BamHI (9010)
SphI (9230)
tetA
ColE1 ori
traF
AvaI (11435)
NotI (11689)
oriV
AvaI (12347)
kilA
NPTIII
IS1
PstI (13505)
NPTIII
trfA
NdeI (15878)
AvaI (16219)
NcoI (16406)
tetA
NcoI (16934)
Left Border
lacI
AvaI (17957)
M13 ori
nos3'
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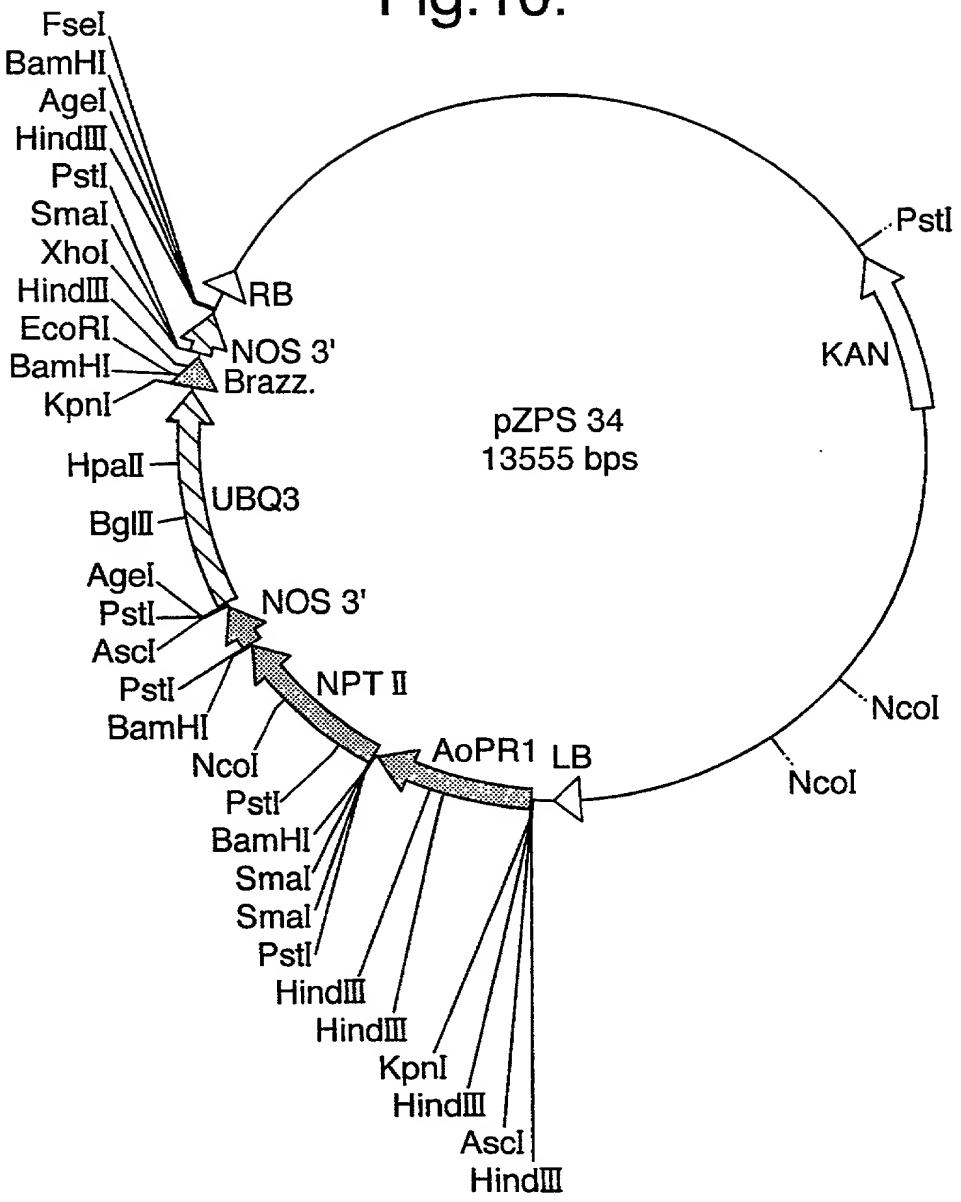
PG-TER

Fig.10.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AgeI
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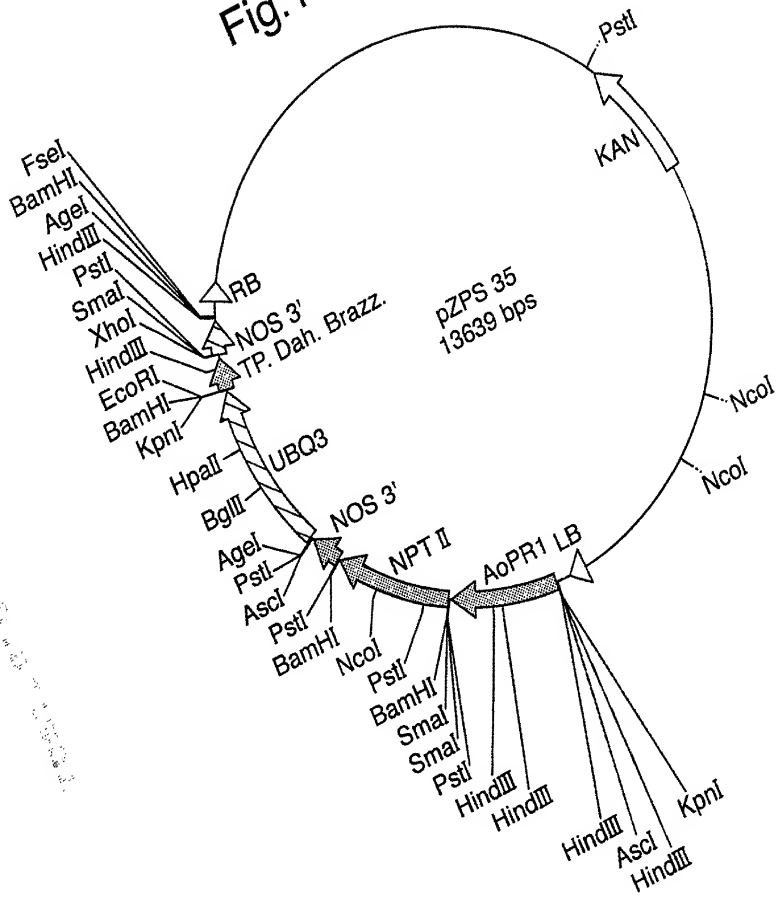
NOS 3'
Brazz.
HpaII
UBQ3
BglII
AgeI
PstI
AscI
PstI
BamHI
NcoI
PstI
BamHI
SmaI
SmaI
PstI
HindIII
HindIII
KpnI
HindIII
AscI
HindIII
NOS 3'
NPT II
AoPR1
LB
pZPS 34
13555 bps
PstI
KAN
NcoI
NcoI

Fig.11.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XhoI
HindIII
EcoRI
BamHI
KpnI
RB
NOS 3'
TP. Dah. Brazz.
HpaII
UBQ3
BglII
NOS 3'
AgeI
PstI
AscI
PstI
BamHI
NcoI
NPT II
AoPR1 LB
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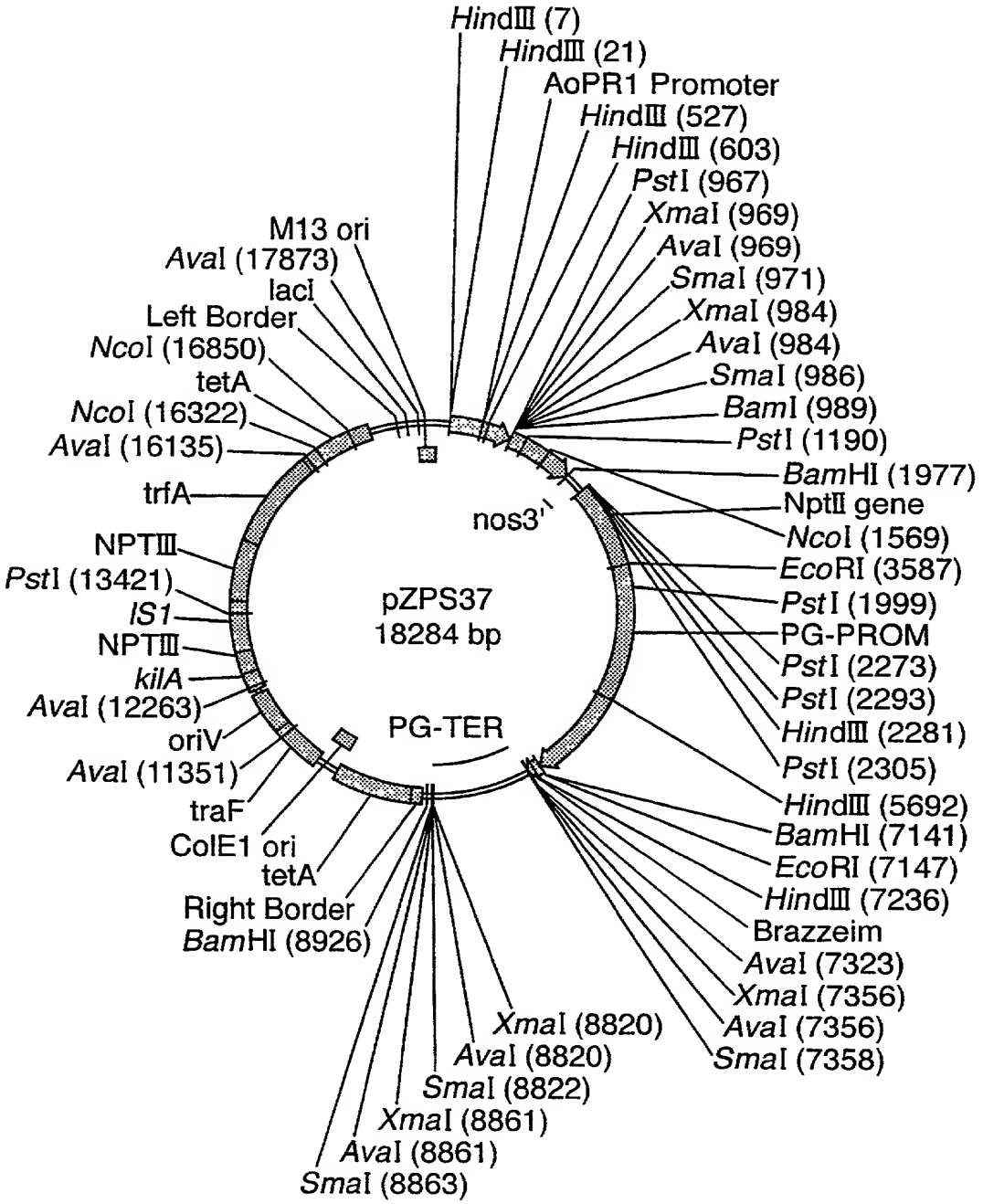
Fig.12.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BamI (989)
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EcoRI (7147)
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SmaI (8863)
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BamHI (8926)
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ColE1 ori
traF
AvaI (11351)
oriV
AvaI (12263)
kilA
NPTIII
IS1
PstI (13421)
NPTIII
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NcoI (16322)
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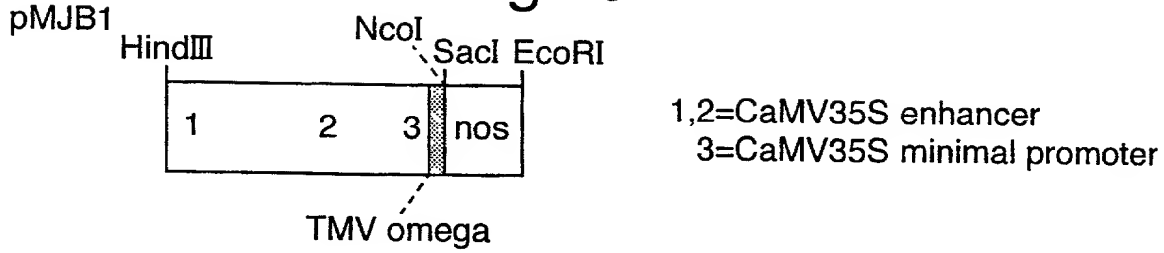
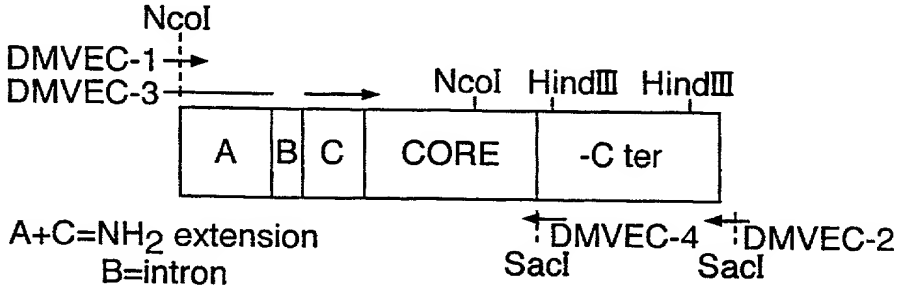
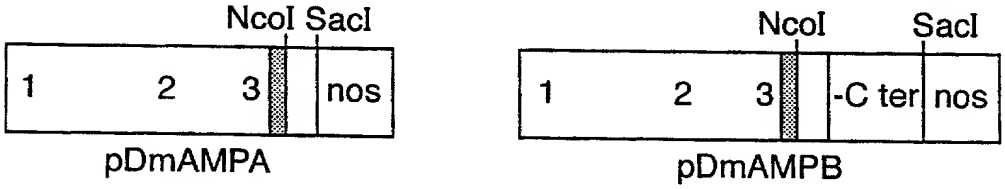
Fig.13.



Structure of DmAMP1 Gene and position of vector construction oligonucleotides



PCR Dahlia genomic DNA with DMVEC-1 and DMVEC-2, isolate 450 bp product.
PCR 450 bp DMVEC-1/DMVEC-2 PCR product with DMVEC 1 and 4.
Isolate 60 bp NcoI / SacI fragment, clone into pMJB1 NcoI / SacI=pDmAMPA.
Cut 450 bp DMVEC-1/DMVEC-2 PCR product NcoI / SacI . Isolate 180 bp NcoI / SacI fragment, clone into pMJB1 NcoI / SacI =pDmAMPB



PCR 450 bp DMVEC-1/DMVEC-2 PCR product with DMVEC 3 and 4.
Isolate 150 bp NcoI fragment, clone into pDmAMPA
and pDmAMPB NcoI =pDmAMPD and pDmAMPE

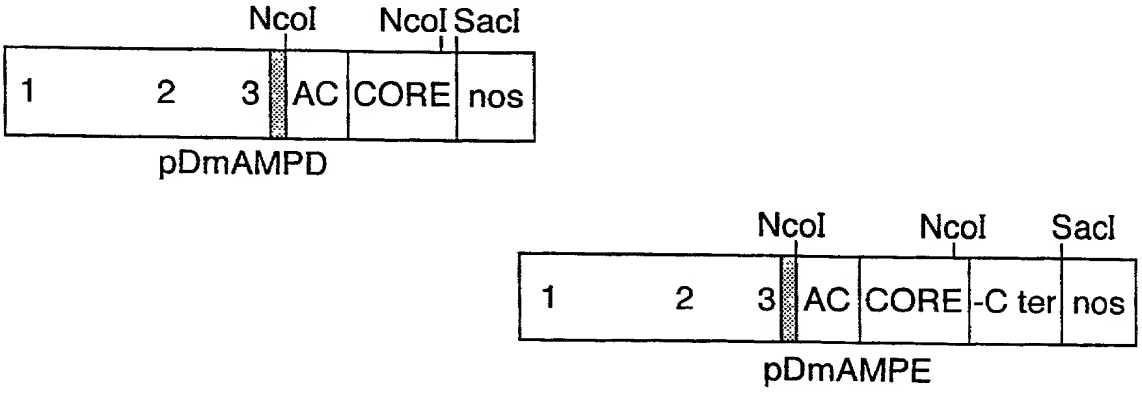


Fig.14.

Sequence ID No.6 Dm-AMP1

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TGG GAG GGA GCT GCT CAT GGA GCT TGC CAT GTT AGA AAC

Sequence ID No.7 Dm-AMP2

GAG GTT TGC GAG AAG GCT TCT AAG ACT TGG TCT GGA AAC

Fig.14 (Cont).

```
TGC GGA AAC ACT GGA CAT TGC GAT AAC CAA TGC AAG TCT

GGA AAG CAT ATG TGC TTC TGC TAC TTC AAC TGC

TGC GGA AAC ACT GGA CAT TGC ... ... ...
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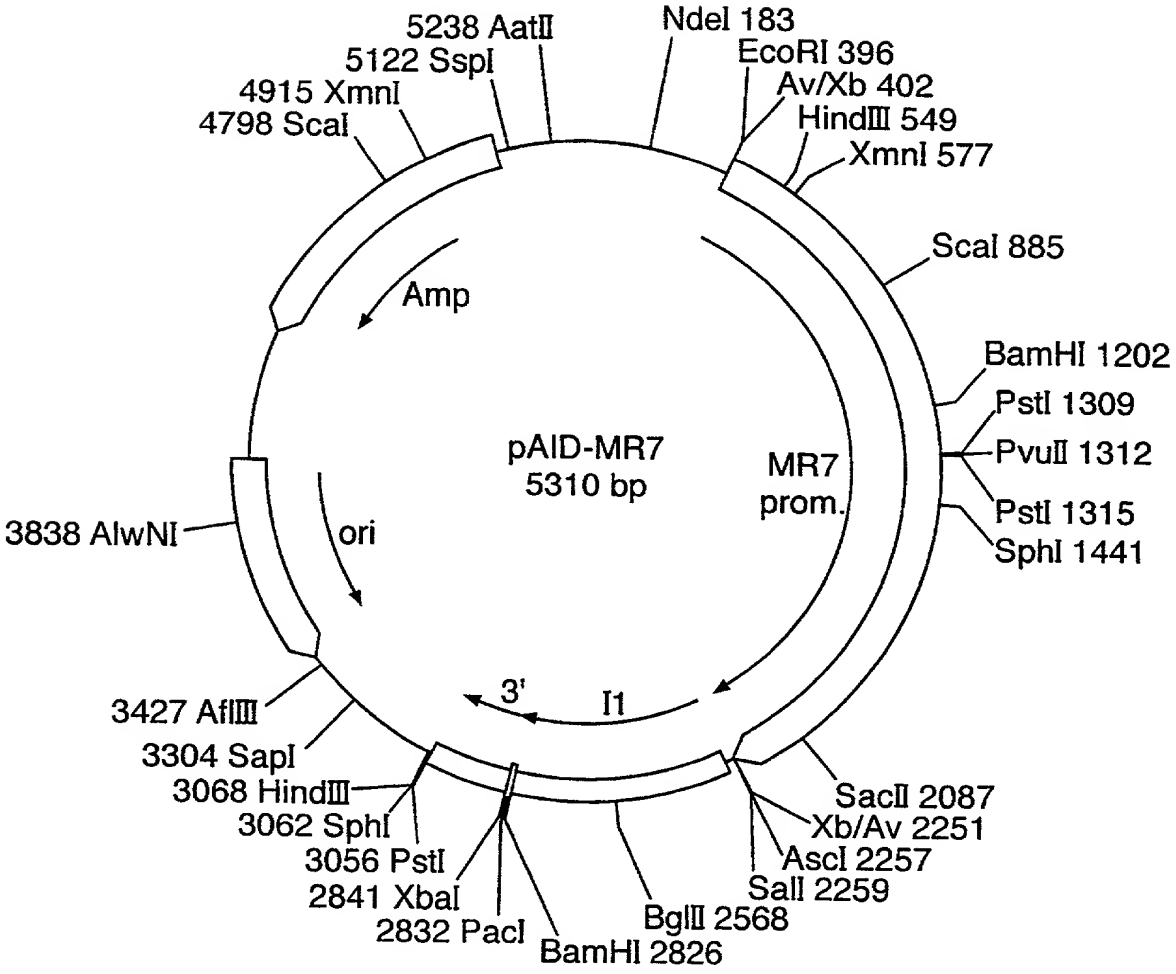
Fig.15.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prom.
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SphI 1441
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Xb/Av 2251
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AscI 2257
3056 PstI
SalI 2259
2841 XbaI
BglII 2568
2832 PacI
BamHI 2826

#3

Docket No.
PPD 50355/UST

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

POLYNUCLEOTIDE SEQUENCES

the specification of which

_____ is attached hereto
__X__ was filed on 17th August 1999 as PCT International Application Number PCT/GB99/02720 and was amended on _____(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Number	Country	Filing Date	Priority Claimed Yes	No
9818003.7	United Kingdom	18th August 1998	X	

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT International Application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

Application Number	Filing Date	Status

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:
Thomas R. Savitsky, Reg No. 31,661; Dianne Burkhard, Reg. No. P41,650;
Liza D. Hohenschutz, Reg. No. 33,712; William E. Dickheiser, Reg. No. 30,169.

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Address all correspondence to: ZENECA Ag Products
Intellectual Property Department
1800 Concord Pike
P.O. Box 15458
Wilmington, DE 19850-5458

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name) 1-00 Ian Jeffrey EVANS

Inventor's signature _____ Date 27th FEBRUARY 2001

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Post Office Address Zeneca Limited, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6ET, United Kingdom

Full name of second inventor, if any (given name, family name) 2-00 John Anthony RAY

Inventor's signature _____ Date 27 FEBRUARY 2001

Residence Bracknell, United Kingom British GBX

Post Office Address Zeneca Limited, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6ET, United Kingdom

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Evans, et al.

Serial No.: 09/763,019

Examiner: TBA

Filed: Filing Receipt Not Received

Entitled: **Polynucleotide Sequences**

Group Art Unit: TBA

Attorney Docket No.: SYN-072

Director of Patents
Washington, D.C. 20231

CERTIFICATE OF FIRST CLASS MAILING UNDER 37 CFR § 1.8

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Date: July 19, 2001

Patricia Corrigan
Patricia Corrigan

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Sir or Madam:

Please recognize each of the following as an Associate Attorney in this case.

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Steven D. Barrett	40,903	James B. Lampert	24,564
Michael J. Bevilacqua	31,091	Keum J. Park	42,059
Nancy Chiu, Ph.D.	43,545	Jason A. Reyes	41,513
Michael A. Diener	37,122	Gretchen A. Rice, Ph.D.	37,429
Peter M. Dichiara	38,005	Donald R. Steinberg	37,241
Richard A. Goldenberg	38,895	Colleen Superko	39,850
Edward D. Grieff	38,898	Hall C. Swaim	22,838
Sally Byrne	40,545	Rajesh Vallabh	35,761
Wayne M. Kennard	30,271	Henry N. Wixon	32,073
David J. Cerveny	44,600	Ayla A. Lari	43,739
Nels Lippert	25,888	Dominic Massa	44,905
Gregory S. Discher	42,488	Irah H. Donner	35,120

Robert McIssac	P46,918	Luke Yeh	43,296
David Cerveney	44,600	David Cavanaugh	36,476
Irah H. Donner	35,120	Gregory S. Discher	42,488
Anthony Kahng	42,704	Maria Maebius	42,967
Cynthia Nicholson	36,880	Tamara Pertmer	P4,856
MaryRose Scozzafava	36,268	Victor Souto	33,458
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Fax: 617-526-5000

Kindly note that the Attorney Docket Number has changed to SYN-072.

Respectfully submitted,

Date: July 13, 2001

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2 Righter Parkway
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Wilmington, DE 19850-5458
Tel: 302-476-2092
Fax: 302-476-2022

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Approved for use through 10/31/2002. OMB 0651-0035
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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Washington, D.C. 20231

Application Number	09/763,019
Filing Date	Filing Receipt Not Recv'd
First Named Inventor	Evans
Group Art Unit	TBA
Examiner Name	TBA
Attorney Docket Number	SYN-072

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[ ] Assignee of record of the entire interest.
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[✓] Attorney or Agent of record.

[ ] Registered practitioner named in the application transmittal letter in an application without an executed oath or declaration. See 37 CFR 1.33(a)(1). Registration Number _____

Typed or Printed Name: Colleen Superko, Reg. No. 39,850

Signature: Colleen Superko

Date: July 19, 2001

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[ ] *Total of _____ forms are submitted.

SEQUENCE LISTING

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Evans, Ian J
Ray, John A

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Arg His Cys Asp Asp Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly
                 50                  55                  60

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Ala Cys His Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn
             65                  70                  75

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Val Leu Ala Ile Ser
20

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                                                        Asp Ile

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			20					25					30		
Lys	Ala	Ser	Lys	Thr	Trp	Ser	Gly	Asn	Cys	Gly	Asn	Thr	Arg	His	Cys
		35					40					45			
Asp	Asp	Gln	Cys	Lys	Ser	Trp	Glu	Gly	Ala	Ala	His	Gly	Ala	Cys	His
	50					55					60				
Val	Arg	Gly	Gly	Lys	His	Met	Cys	Phe	Cys	Tyr	Phe	Asn	Cys	Pro	Lys
65					70					75					80
Ala	Gln	Lys	Leu	Ala	Glu	Asp	Lys	Leu	Arg	Ala	Ala	Glu	Leu	Ala	Lys
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Glu	Lys	Asn	Asn	Ile	Gly	Ala	Glu	Lys	Val	Pro	Ser	Ala	Thr	Pro	
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<213> Dahlia merckii

<400> 9

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            20                  25                  30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Arg His Cys
        35                  40                  45

Asp Asp Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
    50                  55                  60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Ser Lys
65                  70                  75                  80

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Glu Lys Ser Glu Ala Glu Lys Val Pro Ala Thr Pro
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 1               5                  10                  15
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20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Ile Thr Ser His Cys
35 40 45

Asp Asn Gln Cys Arg Ser Trp Glu Gly Ala Ile His Gly Ala Cys His
50 55 60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Ser Lys
65 70 75 80

Ala Asp Glu Leu Ala Lys Glu Lys Ile Glu Ala Glu Lys Met Pro Ala
85 90 95

Thr Pro

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<213> Dahlia merckii

<400> 11
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1 5 10 15

Val Leu Ala Ile Ser Asp Ile Thr Ser Val Arg Gly Glu Val Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
35 40 45

Asp Asn Gln Cys Lys Tyr Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Lys Cys Pro Lys
65 70 75 80

Ala Glu Lys Leu Ala Gln Asp Lys Val Asn Ala Gln Glu Leu Asp Arg
85 90 95

Asp Ala Lys Lys Val Ile Pro Asn Val Glu His Pro
100 105

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<213> Dahlia merckii

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1 5 10 15

Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly Glu Leu Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
35 40 45

Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Lys Lys
65 70 75 80

Ala Glu Lys Leu Ala Gln Asp Lys Leu Lys Ala Glu Gln Leu Ala Gln
85 90 95

Asp Lys Leu Asn Ala Gln Lys Leu Asp Arg Asp Ala Lys Lys Val Val
100 105 110

Pro Asn Val Glu His Pro
115

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<213> Dahlia merckii

<400> 13
Cys Glu Lys Ala Ser Lys Thr Trp
1 5

<210> 14
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<400> 14
Met Cys Phe Cys Tyr Phe Asn Cys
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gagggtgcgg cccatggagc gtgtcatgtg cgtaatggga aacacatgtg tttctgctac 120
ttcaac 126

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<213> Dahlia merckii

<400> 16

Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys Asp Asn Gln
1 5 10 15

Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His Val Arg Asn
20 25 30

Gly Lys His Met Cys Phe Cys Tyr Phe Asn
35 40

<210> 17
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<400> 17
Met Xaa Xaa Xaa Ser Val Ala Phe
1 5

<210> 18
<211> 7
<212> PRT
<213> Dahlia merckii

<400> 18
Asn Gly Lys His Met Cys Phe
1 5

<210> 19
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<400> 19
Gly Ala Cys His Val Arg Asn
1 5

<210> 20
<211> 24
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Oligonucleotide

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tgyganaang cnwsnaarac ntgg

<210> 21
<211> 24
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<220>
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<222> (9, 12, 15)
<223> n is any residue

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Oligonucleotide

<400> 21
carttraant ancanaaarc acat 24

<210> 22
<211> 23
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<223> n is any residue

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Oligonucleotide

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<210> 23
<211> 20
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Oligonucleotide

<400> 23
aaacacatgt gtttcccatt 20

<210> 24
<211> 19
<212> DNA
<213> Artificial Sequence

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Oligonucleotide

<400> 24
agcgtgtcat gtgcgtaat 19

<210> 25
<211> 23
<212> DNA
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Oligonucleotide

<400> 25
taaagaaacc gacccttca cgg 23

<210> 26
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aaaccgaccg agctcacgga tgttcaacgt ttggaac 37

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tcgccatctc agatatcgca tccgttagtg gagaactatg cgagaaa 107

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agcaagcttt tcgggagctc aacaattgaa gtaa 34

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<400> 30
aggaagttca tttcatttgg 20

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<220>
<223> Description of Artificial Sequence: Primer

<400> 31
gcctttggca caacttctgc ctctttccga tgagttgttc ggctttaagt ttgtc 55

<210> 32
<211> 29
<212> DNA
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<220>
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ttagagctcc tattaacaag gaaagtagc 29

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Ile Gly Lys Arg
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<222> (76)..(525)

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Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val
1 5 10

ctg atc ctt ttc gtg ctc gcc atc tca gat atc gca tcc gtt agt gga 159
Leu Ile Leu Phe Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly
15 20 25

gaa cta tgc gag aaa gct agc aag acg tgg tcg ggc aac tgt ggc aac 207
Glu Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn
30 35 40

acg gga cat tgt gac aac caa tgt aaa tca tgg gag ggt gcg gcc cat 255
Thr Gly His Cys Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His
45 50 55 60

gga gcg tgt cat gtg cgt aac ggg aaa cac atg tgt ttc tgt tac ttc 303
Gly Ala Cys His Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe
65 70 75

aat tgt aaa aaa gcc gaa aag ctt gct caa gac aaa ctt aaa gcc gaa 351
Asn Cys Lys Lys Ala Glu Lys Leu Ala Gln Asp Lys Leu Lys Ala Glu
80 85 90

caa ctc atc gga aag agg cag aag ttg tgc caa agg cca agt ggg aca 399
Gln Leu Ile Gly Lys Arg Gln Lys Leu Cys Gln Arg Pro Ser Gly Thr
95 100 105

tgg tca gga gtc tgt gga aac aat aac gca tgc aag aat cag tgc att 447
Trp Ser Gly Val Cys Gly Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile
110 115 120

aga ctt gag aaa gca cga cat gga tct tgc aac tat gtc ttc cca gct 495
Arg Leu Glu Lys Ala Arg His Gly Ser Cys Asn Tyr Val Phe Pro Ala
125 130 135 140

cac aag tgt atc tgc tac ttt cct tgt taa taggagctc 534
His Lys Cys Ile Cys Tyr Phe Pro Cys
145 150

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1 5 10 15

Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly Glu Leu Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
35 40 45

Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Lys Lys
65 70 75 80

Ala Glu Lys Leu Ala Gln Asp Lys Leu Lys Ala Glu Gln Leu Ile Gly
85 90 95

Lys Arg Gln Lys Leu Cys Gln Arg Pro Ser Gly Thr Trp Ser Gly Val
100 105 110

Cys Gly Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Arg Leu Glu Lys
115 120 125

Ala Arg His Gly Ser Cys Asn Tyr Val Phe Pro Ala His Lys Cys Ile
130 135 140

Cys Tyr Phe Pro Cys
145

<210> 36
<211> 316
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<213> Artificial Sequence

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atttacaatt acacc atg gtg aat cgg tcg gtt gcg ttc tcc gcg ttc gtt 111
Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val
1 5 10

ctg atc ctt ttc gtg ctc gcc atc tca gat atc gca tcc gtt agt gga 159
Leu Ile Leu Phe Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly
15 20 25

gaa cta tgc gag aaa gct agc aag acg tgg tcg ggc aac tgt ggc aac 207
Glu Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn
30 35 40

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acg gga cat tgt gac aac caa tgt aaa tca tgg gag ggt gcg gcc cat   255
Thr Gly His Cys Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His
 45                  50                  55                  60

gga gcg tgt cat gtg cgt aat ggg aaa cac atg tgt ttc tgt tac ttc   303
Gly Ala Cys His Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe
                 65                  70                  75

aat tgt tga gctc                                                 316
Asn Cys
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<210> 37
<211> 78
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic sequence

<400> 37

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Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val Leu Ile Leu Phe
  1               5                  10                  15

Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly Glu Leu Cys Glu
             20                  25                  30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
         35                  40                  45

Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
     50                  55                  60

Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys
 65                  70                  75
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Ray, John

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aaa aat tca gtt gct ttc ttt gca ttg tgc ctg ctt ctt ttc att ctt  106
Lys Asn Ser Val Ala Phe Phe Ala Leu Cys Leu Leu Leu Phe Ile Leu
          5                  10                  15

gct atc tca gaa atc aga tcg gtg aag ggg gaa tta tgt gag aag gca  154
Ala Ile Ser Glu Ile Arg Ser Val Lys Gly Glu Leu Cys Glu Lys Ala
     20                  25                  30

agc aag aca tgg tct gga aat tgt ggc aat aca aga cac tgt gat gac  202
Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Arg His Cys Asp Asp
 35                  40                  45                  50

cag tgc aag tct tgg gag ggt gca gcc cat gga gct tgt cac gtg cgc  250
Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His Val Arg
                 55                  60                  65

ggt ggg aaa cac atg tgc ttc tgc tac ttc aac tgt ccc aaa gcc cag  298
Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Pro Lys Ala Gln
             70                  75                  80

aag ttg gct gag gat aaa ctc aga gca gca gag cta gca aag gag aag  346
Lys Leu Ala Glu Asp Lys Leu Arg Ala Ala Glu Leu Ala Lys Glu Lys
         85                  90                  95
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aat aat att gga gct gaa aag gtg cct tca gcc aca cct tga          388
Asn Asn Ile Gly Ala Glu Lys Val Pro Ser Ala Thr Pro
    100                 105                 110

gtactaacaa a                                                    399
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           Met Ala Lys Asn Ser Val Ala Phe Leu Ala Phe Leu Leu
             1               5                  10

ctt ctt ttc gtt ctt gct atc tca gaa atc gga tcg gtg aag ggg gaa   97
Leu Leu Phe Val Leu Ala Ile Ser Glu Ile Gly Ser Val Lys Gly Glu
     15                  20                  25

tta tgt gag aag gca agc aag aca tgg tct gga aat tgt ggc aat aca   145
Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr
 30                  35                  40                  45

aga cac tgt gat gac cag tgc aag tct tgg gag ggc gca gcc cat gga   193
Arg His Cys Asp Asp Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly
                 50                  55                  60

gct tgt cac gtg cgc ggt ggg aaa cac atg tgc ttt tgc tac ttc aac   241
Ala Cys His Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn
             65                  70                  75

tgt tcc aaa gcc cag aag ctg gct cag gat aaa ctc aaa gcc gac aag   289
Cys Ser Lys Ala Gln Lys Leu Ala Gln Asp Lys Leu Lys Ala Asp Lys
         80                  85                  90

ctc gcc aag gag aag agt gaa gcc gaa aag gtg cca gct aca cct tga   337
Leu Ala Lys Glu Lys Ser Glu Ala Glu Lys Val Pro Ala Thr Pro
     95                  100                 105

gtactaacaa gtgttgtatg attatgaata aagagaaaat gctttctagt taccatattt 397

agcattctct aatgtgtaat gtttgttgct tttggaacta attgcttaac tatgattcca 457

gctaataatg ttttaagtat ataatataag ttatcttatt ttgaagcctg taaaaaaaaa 517

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<213> Dahlia merckii

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Met Ala Lys Asn Ser Val Ala Phe Phe Ala
1 5 10

ttt gtc ctg ctt ctt ttc gtt ctt gct atc tca gaa att gga tcg gtg 101
Phe Val Leu Leu Leu Phe Val Leu Ala Ile Ser Glu Ile Gly Ser Val
15 20 25

aag gga gaa tta tgt gag aag gca agc aag aca tgg tct gga aat tgt 149
Lys Gly Glu Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys
30 35 40

ggc atc aca tca cac tgt gac aac cag tgc cgg tcg tgg gag ggt gca 197
Gly Ile Thr Ser His Cys Asp Asn Gln Cys Arg Ser Trp Glu Gly Ala
45 50 55

atc cat gga gct tgt cac gtg cgc ggt ggg aaa cac atg tgc ttc tgc 245
Ile His Gly Ala Cys His Val Arg Gly Gly Lys His Met Cys Phe Cys
60 65 70

tac ttc aac tgt tcc aaa gcc gat gag ctc gcg aag gag aag att gaa 293
Tyr Phe Asn Cys Ser Lys Ala Asp Glu Leu Ala Lys Glu Lys Ile Glu
75 80 85 90

gcc gaa aag atg cca gcc aca cct tga gtactaacaa atgctatatg 340
Ala Glu Lys Met Pro Ala Thr Pro
95

attataaata aagagaaaat gctttctaaa aaaaaaaaaa aaaaa 385

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Met Val Asn Arg Ser Val Ala Phe Ser Val Phe
1 5 10

gtt ctg atc ctt ttc gtg ctc gcc atc tca gat atc aca agt gtg aga 100
Val Leu Ile Leu Phe Val Leu Ala Ile Ser Asp Ile Thr Ser Val Arg
15 20 25

gga gaa gta tgc gag aaa gct agc aag aca tgg tca gga aac tgt ggc 148
Gly Glu Val Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly
30 35 40

aac acg gga cac tgt gac aac caa tgt aaa tac tgg gag ggg gcg gcc 196
Asn Thr Gly His Cys Asp Asn Gln Cys Lys Tyr Trp Glu Gly Ala Ala
45 50 55

cat ggg gcg tgc cac gtg cgt gga ggg aaa cac atg tgt ttc tgc tac 244
His Gly Ala Cys His Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr
60 65 70 75

ttc aag tgt ccc aaa gcc gaa aag ctt gct caa gac aaa gtt aat gcc 292
Phe Lys Cys Pro Lys Ala Glu Lys Leu Ala Gln Asp Lys Val Asn Ala
80 85 90

caa gag ctt gac cgt gat gcc aag aaa gtg att ccg aac gtt gaa cat 340
Gln Glu Leu Asp Arg Asp Ala Lys Lys Val Ile Pro Asn Val Glu His
95 100 105

ccg tga aagggtcggt ttctttaaat agaaagtctt agattacgaa tgcgaataac 396
Pro

tatagaaaat gtttgctaaa tgtcacatta taattagaac tttatgattg ttgtcaatag 456

ggcattttct tgttagtgat atgtgtaata aggtgatgct tttatgcttt tcgtgcgtaa 516

gagttttcga ctatgtgtaa taaagaaagg gtctttttt tttaaaaaaa aaaaaaaaaa 576

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<213> Dahlia merckii

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tcaggttatc aaatctttag ttcatttatt gaatatgata gtatttatat tcttttatgg 120
ttttatgtgt tctgacaagt tgcaaatatt gagtagatat cgcatccgtt agtggagaac 180
tatgcgagaa agctagcaag acatggtcgg gaaactgtgg caatacggga cattgtgaca 240
accaatgtaa atcatgggag ggtgcggccc atggagcgtg tcatgtgcgt aacgggaaac 300
acatgtgttt ctgttacttc aattgtaaaa aagccgaaaa gcttgctcaa gacaaactta 360
aagccgaaca actcgctcaa gacaaactta atgcccaaaa gcttgaccgt gatgccaaga 420
aagtggttcc aaacgttgaa catccg 446

<210> 6
<211> 150
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<213> Dahlia merckii

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gataaccaat gcaagtcttg ggagggagct gctcatggag cttgccatgt tagaaacgga 120
aagcatatgt gcttctgcta cttcaactgc 150

<210> 7
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gaggtttgcg agaaggcttc taagacttgg tctggaaact gcggaaacac tggacattgc 60

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Met Ala Lys Asn Ser Val Ala Phe Phe Ala Leu Cys Leu Leu Leu Phe
1 5 10 15

Ile Leu Ala Ile Ser Glu Ile Arg Ser Val Lys Gly Glu Leu Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Arg His Cys
35 40 45

Asp Asp Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Pro Lys
65 70 75 80

Ala Gln Lys Leu Ala Glu Asp Lys Leu Arg Ala Ala Glu Leu Ala Lys
85 90 95

Glu Lys Asn Asn Ile Gly Ala Glu Lys Val Pro Ser Ala Thr Pro
100 105 110

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Met Ala Lys Asn Ser Val Ala Phe Leu Ala Phe Leu Leu Leu Leu Phe
1 5 10 15

Val Leu Ala Ile Ser Glu Ile Gly Ser Val Lys Gly Glu Leu Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Arg His Cys
35 40 45

Asp Asp Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Ser Lys
65 70 75 80

Ala Gln Lys Leu Ala Gln Asp Lys Leu Lys Ala Asp Lys Leu Ala Lys
85 90 95

Glu Lys Ser Glu Ala Glu Lys Val Pro Ala Thr Pro
100 105

<210> 10

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Met Ala Lys Asn Ser Val Ala Phe Phe Ala Phe Val Leu Leu Leu Phe
1 5 10 15

Val Leu Ala Ile Ser Glu Ile Gly Ser Val Lys Gly Glu Leu Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Ile Thr Ser His Cys
35 40 45

Asp Asn Gln Cys Arg Ser Trp Glu Gly Ala Ile His Gly Ala Cys His
50 55 60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Ser Lys
65 70 75 80

Ala Asp Glu Leu Ala Lys Glu Lys Ile Glu Ala Glu Lys Met Pro Ala
85 90 95

Thr Pro

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<400> 11
Met Val Asn Arg Ser Val Ala Phe Ser Val Phe Val Leu Ile Leu Phe
1 5 10 15

Val Leu Ala Ile Ser Asp Ile Thr Ser Val Arg Gly Glu Val Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
35 40 45

Asp Asn Gln Cys Lys Tyr Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Gly Gly Lys His Met Cys Phe Cys Tyr Phe Lys Cys Pro Lys
65 70 75 80

Ala Glu Lys Leu Ala Gln Asp Lys Val Asn Ala Gln Glu Leu Asp Arg
85 90 95

Asp Ala Lys Lys Val Ile Pro Asn Val Glu His Pro
100 105

<210> 12
<211> 118
<212> PRT
<213> Dahlia merckii

<400> 12
Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val Leu Ile Leu Phe
1 5 10 15

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Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly Glu Leu Cys Glu
             20                  25                  30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
         35                  40                  45

Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
     50                  55                  60

Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Lys Lys
 65                  70                  75                  80

Ala Glu Lys Leu Ala Gln Asp Lys Leu Lys Ala Glu Gln Leu Ala Gln
                 85                  90                  95

Asp Lys Leu Asn Ala Gln Lys Leu Asp Arg Asp Ala Lys Lys Val Val
             100                 105                 110

Pro Asn Val Glu His Pro
         115
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<210> 13
<211> 8
<212> PRT
<213> Dahlia merckii

<400> 13

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Cys Glu Lys Ala Ser Lys Thr Trp
  1               5
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<210> 14
<211> 8
<212> PRT
<213> Dahlia merckii

<400> 14

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Met Cys Phe Cys Tyr Phe Asn Cys
  1               5
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<210> 15
<211> 126
<212> DNA
<213> Dahlia merckii

<400> 15

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aagacgtggt cgggaaactg tggcaatacg ggacattgtg acaaccaatg taaatcatgg 60
gagggtgcgg cccatggagc gtgtcatgtg cgtaatggga aacacatgtg tttctgctac 120
ttcaac                                                            126
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<210> 16
<211> 42
<212> PRT
<213> Dahlia merckii

<400> 16
Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys Asp Asn Gln
1 5 10 15

Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His Val Arg Asn
20 25 30

Gly Lys His Met Cys Phe Cys Tyr Phe Asn
35 40

<210> 17
<211> 8
<212> PRT
<213> Dahlia merckii

<220>
<221> SITE
<222> (2)
<223> Xaa is Ala or Val

<220>
<221> SITE
<222> (3)
<223> Xaa is Lys or Asn

<220>
<221> SITE
<222> (4)
<223> Xaa is Asn or Arg

<400> 17
Met Xaa Xaa Xaa Ser Val Ala Phe
1 5

<210> 18
<211> 7
<212> PRT
<213> Dahlia merckii

<400> 18
Asn Gly Lys His Met Cys Phe
1 5

<210> 19
<211> 7
<212> PRT
<213> Dahlia merckii

<400> 19
Gly Ala Cys His Val Arg Asn
1 5

<210> 20
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<220>
<221> misc_feature
<222> (6)
<223> n is any residue

<220>
<221> misc_feature
<222> (9)
<223> n is any residue

<220>
<221> misc_feature
<222> (12)
<223> n is any residue

<220>
<221> misc_feature
<222> (15)
<223> n is any residue

<220>
<221> misc_feature
<222> (21)
<223> n is any residue

<400> 20
tgyganaang cnwsnaarac ntgg 24

<210> 21
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<220>
<221> misc_feature
<222> (9)
<223> n is any residue

<220>
<221> misc_feature
<222> (12)
<223> n is any residue

<220>
<221> misc_feature
<222> (15)
<223> n is any residue

<400> 21
carttraant ancanaaarc acat 24

<210> 22
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<220>
<221> misc_feature
<222> (9)
<223> n is any residue

<220>
<221> misc_feature
<222> (12)
<223> n is any residue

<220>
<221> misc_feature
<222> (21)
<223> n is any residue

<400> 22
atggcsaanm rntcrgttgc ntt 23

<210> 23
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 23
aaacacatgt gtttcccatt 20

<210> 24
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 24
agcgtgtcat gtgcgtaat 19

<210> 25
<211> 23

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 25
taaagaaacc gacccttca cgg 23

<210> 26
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 26
atcgtagcca tggtgaatcg gtcggttgcg ttctccgcg 39

<210> 27
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 27
aaaccgaccg agctcacgga tgttcaacgt ttggaac 37

<210> 28
<211> 107
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 28
atgcatccat ggtgaatcgg tcggttgcgt tctccgcgtt cgttctgatc cttttcgtgc 60
tcgccatctc agatatcgca tccgttagtg gagaactatg cgagaaa 107

<210> 29
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Oligonucleotide

<400> 29

agcaagcttt tcgggagctc aacaattgaa gtaa 34

<210> 30
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 30
aggaagttca tttcatttgg 20

<210> 31
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 31
gcctttggca caacttctgc ctctttccga tgagttgttc ggctttaagt ttgtc 55

<210> 32
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 32
ttagagctcc tattaacaag gaaagtagc 29

<210> 33
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 33
Ile Gly Lys Arg
1

<210> 34
<211> 534
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (76)..(525)

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 34
ctcgagtatt tttacaacaa ttaccaacaa caacaaacaa caaacaacat tacaattact 60

atttacaatt acacc atg gtg aat cgg tcg gtt gcg ttc tcc gcg ttc gtt 111
Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val
1 5 10

ctg atc ctt ttc gtg ctc gcc atc tca gat atc gca tcc gtt agt gga 159
Leu Ile Leu Phe Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly
15 20 25

gaa cta tgc gag aaa gct agc aag acg tgg tcg ggc aac tgt ggc aac 207
Glu Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn
30 35 40

acg gga cat tgt gac aac caa tgt aaa tca tgg gag ggt gcg gcc cat 255
Thr Gly His Cys Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His
45 50 55 60

gga gcg tgt cat gtg cgt aac ggg aaa cac atg tgt ttc tgt tac ttc 303
Gly Ala Cys His Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe
65 70 75

aat tgt aaa aaa gcc gaa aag ctt gct caa gac aaa ctt aaa gcc gaa 351
Asn Cys Lys Lys Ala Glu Lys Leu Ala Gln Asp Lys Leu Lys Ala Glu
80 85 90

caa ctc atc gga aag agg cag aag ttg tgc caa agg cca agt ggg aca 399
Gln Leu Ile Gly Lys Arg Gln Lys Leu Cys Gln Arg Pro Ser Gly Thr
95 100 105

tgg tca gga gtc tgt gga aac aat aac gca tgc aag aat cag tgc att 447
Trp Ser Gly Val Cys Gly Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile
110 115 120

aga ctt gag aaa gca cga cat gga tct tgc aac tat gtc ttc cca gct 495
Arg Leu Glu Lys Ala Arg His Gly Ser Cys Asn Tyr Val Phe Pro Ala
125 130 135 140

cac aag tgt atc tgc tac ttt cct tgt taa taggagctc 534
His Lys Cys Ile Cys Tyr Phe Pro Cys
145

<210> 35
<211> 149
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 35
Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val Leu Ile Leu Phe
1 5 10 15

Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly Glu Leu Cys Glu
20 25 30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
35 40 45

Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
50 55 60

Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys Lys Lys
65 70 75 80

Ala Glu Lys Leu Ala Gln Asp Lys Leu Lys Ala Glu Gln Leu Ile Gly
85 90 95

Lys Arg Gln Lys Leu Cys Gln Arg Pro Ser Gly Thr Trp Ser Gly Val
100 105 110

Cys Gly Asn Asn Asn Ala Cys Lys Asn Gln Cys Ile Arg Leu Glu Lys
115 120 125

Ala Arg His Gly Ser Cys Asn Tyr Val Phe Pro Ala His Lys Cys Ile
130 135 140

Cys Tyr Phe Pro Cys
145

<210> 36
<211> 316
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (76)..(312)

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 36
ctcgagtatt tttacaacaa ttaccaacaa caacaaacaa caaacaacat tacaattact 60

atttacaatt acacc atg gtg aat cgg tcg gtt gcg ttc tcc gcg ttc gtt 111
Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val
1 5 10

ctg atc ctt ttc gtg ctc gcc atc tca gat atc gca tcc gtt agt gga 159
Leu Ile Leu Phe Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly
15 20 25

gaa cta tgc gag aaa gct agc aag acg tgg tcg ggc aac tgt ggc aac 207
Glu Leu Cys Glu Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn
30 35 40

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acg gga cat tgt gac aac caa tgt aaa tca tgg gag ggt gcg gcc cat   255
Thr Gly His Cys Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His
 45                  50                  55                  60

gga gcg tgt cat gtg cgt aat ggg aaa cac atg tgt ttc tgt tac ttc   303
Gly Ala Cys His Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe
                65                  70                  75

aat tgt tga gctc                                                 316
Asn Cys
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<210> 37
<211> 78
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 37

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Met Val Asn Arg Ser Val Ala Phe Ser Ala Phe Val Leu Ile Leu Phe
  1               5                  10                  15

Val Leu Ala Ile Ser Asp Ile Ala Ser Val Ser Gly Glu Leu Cys Glu
             20                  25                  30

Lys Ala Ser Lys Thr Trp Ser Gly Asn Cys Gly Asn Thr Gly His Cys
         35                  40                  45

Asp Asn Gln Cys Lys Ser Trp Glu Gly Ala Ala His Gly Ala Cys His
     50                  55                  60

Val Arg Asn Gly Lys His Met Cys Phe Cys Tyr Phe Asn Cys
 65                  70                  75
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